VOLUME XIV MAY, 1931 NUMBER 3

JOURNAL OF DAIRY SCIENCE

OFFICIAL ORGAN OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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Itinerary for Members of American Dairy Science Association Western Meeting 1931

THE final completed itinerary of the July meeting shows that this will be an outstanding one from the standpoint of combining sight seeing with business. There will be an excellent opportunity to view scenery and dairying, and to enjoy that equally famous western hospitality. The schedule is as follows:

July 7th Arrive at Los Angeles by 9 A.M. This includes members who come by automobile as well as those who come by train. Visit Moving Picture Studios, Beaches and other points of interest. Luncheon at Beach Club as guests of the Los Angeles Milk Dealers' Association.

July 8th 7 A.M. Those traveling by automobile leave for Merced (specially conducted). Visit oil fields en route.

Those traveling by train continue sightseeing and visiting dairy plants and farms in Los Angeles and vicinity. Leave 6 P.M. for Merced.

July 9th Merced to Yosemite Valley by State Highway. Automobile transportation furnished for those who come by train.

July 10th Leave Yosemite 7 A.M. for Merced, Los Banos, Stanford University, Millbrae Dairy and San Francisco. Automobile transportation furnished.

(Millbrae Dairy—Mills Estate, Inc.—milking 600 pure bred and grade Holsteins. 1500 dairy animals tuberculosis and abortion free. University of California Station experiment in developing healthy dairy cattle from badly infected herd.)

July 11th Sightseeing San Francisco. Instruction in Judging Dairy Products during afternoon.

July 12th Rest—San Francisco.

July 13th Leave San Francisco by automobile for University of California, Berkeley. Points of interest: Greek Theatre, Stadium, International House, Campanile, Library, Agriculture Buildings.

Luncheon on Campus.

Visit Agriculture Extension and Agriculture Economics Departments.

Leave 2:30 P.M. by automobile for University Farm, Davis.

July 14th Formal program—American Dairy Science Association, University Farm, Davis.

July 15th Special trip for Ladies to Lake Tahoe and Sierra Nevada Mountains.

July 16th 8 A.M. Davis to Hartsook Lodge, Redwood Highway. Barbecue lunch en route as guests of the Lake County Farm Bureau. Transportation furnished by members of California Dairy Council.

July 17th Hartsook Lodge to Eureka over Redwood Highway. Transportation furnished by members of California Dairy Council. Visit Dairy Farms and Plants en route. Entertainment by Humboldt County Dairymen's Association.

July 18th Eureka to Grant's Pass over Redwood Highway. Transportation furnished by members of California Dairy Council.

July 19th Grant's Pass, Oregon, to Corvallis by train.

Tours to continue through Oregon, Washington and other Western States and Vancouver, as desired

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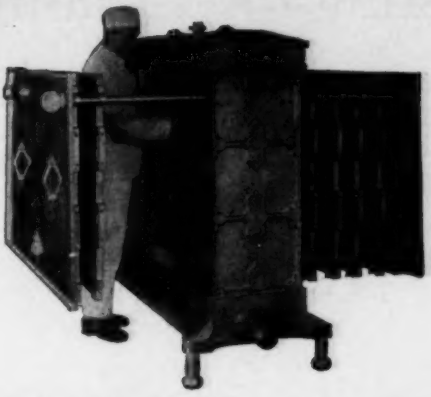

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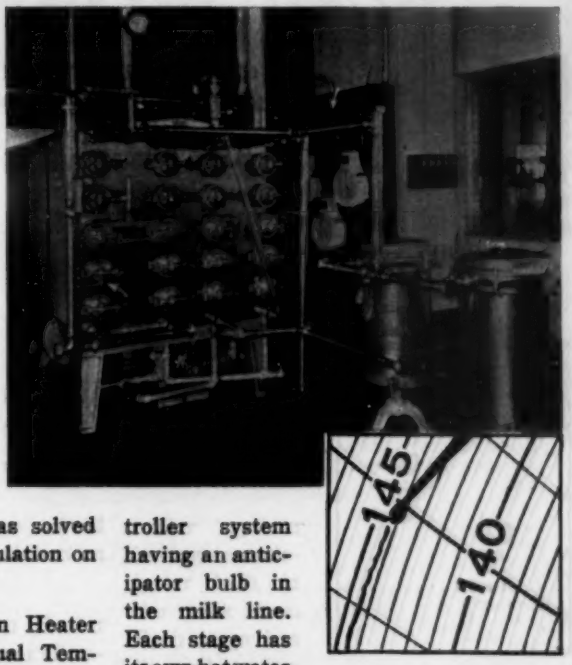

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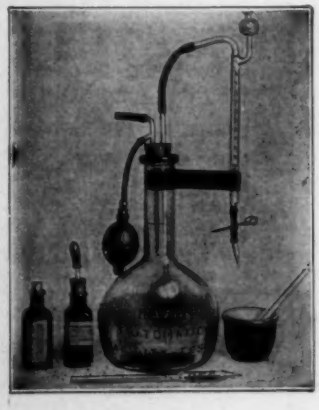

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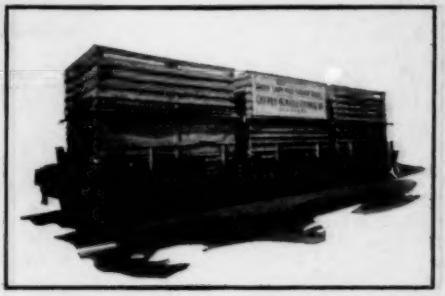

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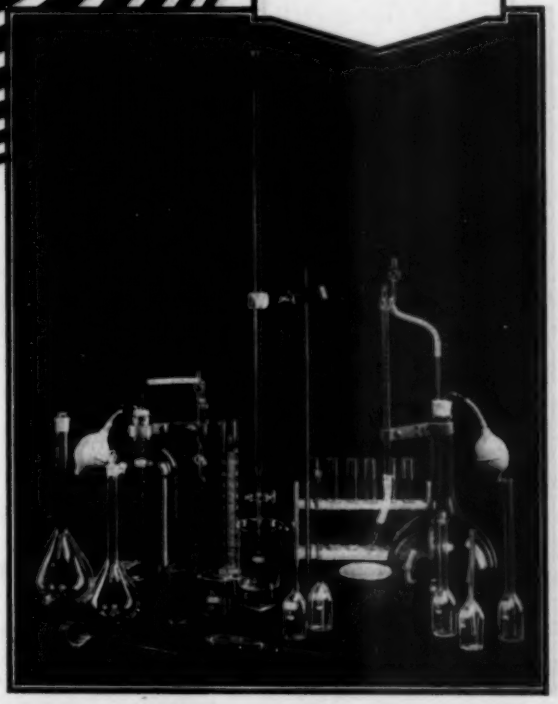

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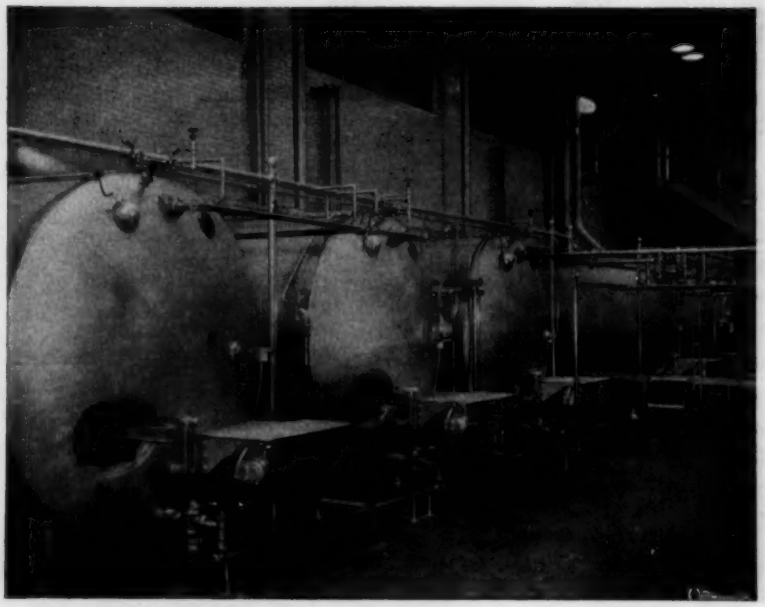

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SKIMMILK AS A FEED FOR DAIRY COWS*

C. H. ECKLES AND E. N. SHULTZ

Division of Dairy Husbandry, University of Minnesota, St. Paul

In the butter-producing areas of the United States the proper utilization of the by-products is an important economic question. Minnesota, for example, with a total butter production of approximately 290,000,000 pounds annually has a total of over six billion pounds of skimmilk and buttermilk available for use on the farms and in the factories. The recommendations of agricultural authorities have been to use the dairy by-products for feeding livestock and the excellent results following this general practice are well known.

In recent years the production of cream for butter manufacture has developed in regions where it is not practical to produce sufficient grain to make swine production profitable on a large scale. The farmer uses all the skimmilk he can as feed for his pigs, calves, and chickens and still often has milk on hand. A calculation based upon census figures shows that after allowing for all the skimmilk that can be used economically by the pigs, calves, and poultry in this state a surplus of over a billion pounds remains. Most of this is not entirely wasted but is used in excessive amounts as feed for livestock. It is well established that the special efficiency of skimmilk in the diet of the growing pig is due to the excellent quality of the protein which supplements the rather low quality proteins of cereals, and to a lesser extent to the mineral and vitamins content. The value per unit of

* Published with approval of the Director as Paper No. 959, Journal Series, Minnesota Agricultural Experiment Station. The data used are taken largely from the thesis of E. N. Shultz submitted in partial fulfillment for the degree of Master of Science, University of Minnesota, June, 1929.

Received for publication August 27, 1930.

skimmilk in the ration rapidly declines when fed in amounts beyond that needed to supplement the grain. In this case the milk solids serve merely as a source of digestible nutrients.

Records taken by the Division of Farm Management and Agricultural Economics of the University of Minnesota from a cost accounting route including 23 farms in Pine County, Minnesota illustrate the situation where a surplus of skimmilk is available.

With a total milk production of approximately 2,000,000 pounds, 328,320 pounds of skimmilk were unaccounted for after feeding the livestock all that could be used. Twenty-three farms on the average fed pigs 1276 pounds of skimmilk for each 100 pound of pork produced. On six out of the 23 farms skimmilk was fed to cows and on three to the horses as well. On most of the farms, curd prepared as in the manufacture of cottage cheese, was fed poultry as a means of increasing the consumption. The uneconomical use of the skimmilk is shown especially by the high rate of feeding to swine. As a result of feeding trials by various investigators it appears well established that the most economical use of skimmilk or buttermilk is by feeding it at the rate of about 525 pounds to the hundred of pork produced.

The attempt to find economical uses for skimmilk is further illustrated by a report from the tester for a Herd Improvement Association in Northern Minnesota that 23 out of 26 members were feeding skimmilk to cows as the result of recommendations by the experiment station. The use of skimmilk in this manner appears to be the proper course in view of the situation which makes impractical the raising of sufficient swine to consume the by-products available and the further fact that the character of the crops grown are such that it is necessary on most farms to buy high protein concentrates to supplement the farm grown feeds.

The alternative to some such disposal is the organization of marketing units to handle whole milk and to utilize the by-products for commercial purposes. This may be the ultimate solution but until this comes about the skimmilk can be used to advantage on the farm in place of high protein concentrates.

Eight pounds of average skimmilk contains almost exactly the

same amount of digestible crude protein and total digestible nutrients as one pound of linseed oilmeal. The high biological value of milk protein is well known. On these grounds eight pounds of skimmilk should equal in feeding value one pound of linseed meal. The Dairy Division of the University of Minnesota has recommended that skimmilk be fed to cows when sufficient numbers of growing pigs, calves, or poultry are not available to utilize it in the usual manner.

In making this recommendation the fact was not overlooked that some objection to the practice may be raised from the disease standpoint, more especially in connection abortion disease. The opinion of the experiment station veterinarians is, that although this question may be raised with good reason, it would not seem especially serious in view of the fact that where the disease is present in a herd the members are subject to possible infection in other ways and the feeding of milk is not for this reason particularly significant.

Although in some sections the feeding of skimmilk to cows is by no means an uncommon practice, very little experimental data are available dealing with the subject. Beach and Clark of the Storrs Experiment Station (1) reported a feeding trial with 24 cows, although only 4 of the number would drink the skimmilk. In the trial using three animals, and nine checks skimmilk was substituted for half the grain at the rate of eight pounds to one of grain. The milk production was approximately 6 per cent greater during the period when skimmilk was fed.

Arnett (2) conducted an eighty-four day trial with two groups of 4 cows each. The two groups received the same amount of hay, silage, and grain and one group 20 pounds of skimmilk daily in addition. The group receiving the skimmilk produced approximately 10 per cent more milk. In neither of these experiments was skimmilk used primarily as a protein supplement which is clearly the place it should fill in the ration for the most economical results.

Two German workers, Honcamp and Helms (3), recently reported a feeding trial in which skimmilk was compared with a high protein supplement as part of a ration for lactating cows.

Using the reversal plan, eight cows were fed for three periods of twenty days each with ten days preliminary periods. During the first and third periods each cow received 22.5 pounds of skimmilk daily. During the second period the skimmilk was replaced with soybean oilmeal and dried beet pulp on the basis of starch value and digestible protein. The production during the period of skimmilk feeding was essentially the same as for the period of soybean meal feeding. The authors conclude that although skimmilk represents a useful high protein feed, it is best adapted for the feeding of pigs and calves. The market value of the skimmilk was given as twice its feed equivalent in soybean meal and beet pulp. On account of the lower value of skimmilk in the United States this conclusion would not apply here.

Before experimental trials were made it was considered reasonably safe to base recommendations regarding the use of skimmilk as part of the ration for dairy cows upon the well established facts concerning the composition of skimmilk and the nature of the proteins. However, it seemed advisable before going very far, to confirm these conclusions by means of some experimental results. Accordingly tests were arranged to compare the results of replacing linseed oilmeal in the ration with skimmilk on the basis of 8 pounds of skimmilk to one of the meal. A trial was made with lactating cows and another with growing dairy heifers.

In the test with cows 9 animals were used following the double reversal plan in which group A receiving skimmilk in periods 1 and 3, and linseed oilmeal in the second period. Group B received the oilmeal in the first and third periods and skimmilk during the second period. The periods were 24 days in length with a six day transitional period. The grain mixture for both groups consisted of ground corn 350, ground barley 250, ground oats 200, and bran 200 pounds. The roughage was mixed hay and silage. This ration was so selected to require additional protein to meet the needs of the cows. During the skimmilk periods this necessary protein was supplied by feeding skimmilk at the rate of 2 pounds to each pound of the feed mixture used. The oilmeal was fed at the rate of one-fourth pound to each pound of the grain mixture. During the skimmilk periods 27.7

per cent of the digestible crude protein was supplied by the skimmilk. The linseed oilmeal supplied 27 per cent of the protein during the periods when this concentrate was fed.

The amount of the ration to be fed was calculated by means of the Morrison Standard and adjustments in amount were made only during the transition periods. Corn silage was fed at the rate of approximately 3 pounds daily per 100 pounds of live

TABLE 1

Milk and fat production

Skimmilk versus linseed oilmeal as protein supplements

24-DAY PERIODS	PRODUCTION PER COW			GAIN IN WEIGHT PER COW
	Milk	Fat		
	pounds	*per cent*	*pounds*	*pounds*
Group A (3 cows):				
1. Skimmilk, 24 days	691.4	4.15	28.70	26
per day	28.8		1.19	
2. Linseed oilmeal, 24 days	650.8	4.20	27.36	−29
per day	27.1		1.14	
3. Skimmilk, 24 days	577.7	4.16	24.04	24
per day	24.1		1.01	
Group B (4 cows):				
1. Linseed oilmeal, 24 days	671.2	4.12	27.68	18
per day	27.9		1.15	
2. Skimmilk, 24 days	606.0	4.24	25.70	−12
per day	25.2		1.07	
3. Linseed oilmeal, 24 days	570.5	4.25	24.29	21
per day	23.7		1.01	

weight. The cows were fed twice daily and milked with a milking machine. Complete feed records were kept, weights of the cows were taken for three days at the beginning and end of each period, and composite milk samples were analyzed for fat at eight-day intervals. It was found only one of the seven cows would drink the skimmilk. After some preliminary tests the plan was adopted of weighing the grain for a particular cow into a container and

pouring the desired amount of skimmilk on the grain just previous to placing it before the cow. The grain mixture absorbed the milk and no loss of skimmilk occurred when the feed wet with the milk was placed on top of the silage.

Table 1 shows the average milk yield for group A was slightly in favor of linseed meal, for group B the skimmilk period showed a small lead. This difference is considered too small to be significant. It is recognized that the numbers of cows used was rather small but in view of the fact that the results corroborate those which would be expected from the known nutritive values of the feeds, it seems safe to accept them as indicating the value of skimmilk under the condition described. The increase in weights of the animals used was probably the result of a slight over-feeding due to the calculation of the ration being made during the transition periods. When this ration was continued without change during the twenty-four-day period the natural decline in milk production resulted in a small excess of nutrients before the end of the period. The loss in weight for both groups during the second period is accounted for by weather conditions. The experiment was not set up in such a manner as to give indications of a possible greater value for milk productions of the proteins from the skimmilk because the amount of protein was presumably sufficient in the ration received by both groups.

FEEDING TRIALS WITH HEIFERS

The value of skimmilk for feeding the calf up to the usual age of weaning is well known and is not a part of this consideration. It was the purpose to determine if 8 pounds of skimmilk would serve the same purpose in the ration as one pound of linseed meal for animals between the usual age for milk feeding and maturity.

Six heifers were used ranging in age from seven to twelve months divided into two groups. The trial covered 90 days. The roughage was timothy hay of a good grade. With this was fed a small allowance of ground corn supplemented for one group by linseed oilmeal, for the other by skimmilk. The low protein roughage was selected in order that a fairly large amount of protein would

be necessary as a supplement. The results are given in table 2. The comparison in gains is made to the Eckles' normal figures (4).

It will be noted that two calves in each lot greatly exceeded the normal gain while one in each lot fell behind. The average gain of 1.11 pounds per day for the group receiving skimmilk is almost identical with the figure 1.14 from the group fed linseed oilmeal. As in the case of the cows in milk 8 pounds of skimmilk appear to be equal to one pound of linseed oilmeal as a supplement to ordinary rations lacking in protein. It is probable that under certain conditions the higher biological value of milk protein

TABLE 2

Skimmilk versus linseed oilmeal as a supplementary feed for dairy heifers

CALF NUMBER	AGE BEGINNING	WEIGHT BEGINNING	TIMOTHY HAY PER DAY	CORN PER DAY	LINSEED OILMEAL	SKIM MILK PER DAY	GAIN PER DAY	PER CENT NORMAL GAIN
	days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*per cent*
1	332	616	13.6	2.25		18	1.31	177
2	236	523	9.2	2.0		16	1.24	105
3	247	347	6.8	1.8		14	0.79	78
Average							1.11	
4	331	645	13.6	2.25	2.25		1.40	189
5	338	371	8.0	2.0	2.0		1.06	138
6	213	393	7.8	2.0	2.0		0.95	77
Average							1.14	

would widen this ratio. The calves receiving the skimmilk supplement made distinctly greater skeletal growth than the group receiving the oilmeal, but the numbers used were too small to justify any conclusions.

DISCUSSION

The number of animals used in the trials reported was limited but it is felt the results may be accepted in view of the fact that these experimental data confirm the results expected from a comparison of the nutritive values of skimmilk and linseed oilmeal. It is probable with a more careful set up of experimental con-

ditions a higher nutritive quality of milk protein for milk production could be observed. The more rapid skeletal growth of the heifers receiving the skimmilk may be an indication of this factor. In ordinary feeding practice the possibility of better results from the skimmilk due to the quality of the protein could be ignored on account of the limited information available and the difficulties of applying the present knowledge to the complicated situation resulting from the use of mixed rations.

It is clear that the value of skimmilk as a feed for cows will depend largely upon the character of the ration with which it is fed. If fed, as has been done by some farmers, by merely adding to a ration already meeting the requirements of the animals reasonably well the skimmilk serves only as a source of additional calories and protein. Under these conditions of feeding the fairest comparison probably is on the basis of the total digestible nutrients. On this basis with corn at $30 per ton skimmilk would be worth 16 cents per 100 pounds. On a protein basis, if linseed oilmeal costs $55 per ton skimmilk should be worth 33 cents per hundred. To use skimmilk economically as a feed for cattle beyond the usual age for milk feeding requires that the ration be so formulated as to depend upon the skimmilk for a considerable proportion of the protein. It is clear in regions where the feed grown on the farms contain an abundance of protein, or low protein feeds are not cheaper on the market, there is small reason for considering the feeeing of skimmilk to mature animals. The place where it may be done with advantage is in the middle west where a surplus of skimmilk is available on many farms and where the crops grown are such as to require the purchase of high protein concentrates as supplementary feeds.

It is possible the present surplus of skimmilk will disappear in time with a reorganization of the dairy manufacturing units which will result in facilities for using the by-products to better advantage. At present only about 5 per cent of the skimmilk and buttermilk available in Minnesota is used for commerical purposes and the price paid the producer for the product is no more, and is often less, than he can realize by feeding the skimmilk to his farm animals under proper conditions. The use of skimmilk as a

feed for cows and growing heifers extends the possibilities of using the surplus skimmilk to advantage on the farm.

CONCLUSIONS

1. Skimmilk can be used as a protein supplement in the ration for dairy cattle, replacing linseed oilmeal when fed at the rate of 8 pounds of skimmilk to 1 pound of linseed oilmeal.

2. The feeding of skimmilk as a source of protein to dairy cattle is an economical practice in areas where the use of surplus skimmilk is a problem.

3. The value of skimmilk for feeding purposes is determined by the market prices of the high protein concentrates which can be purchased.

4. Skimmilk can be used economically to supply protein in the rations of young dairy stock fed a low protein roughage such as timothy hay.

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THE EFFECT OF ACIDITY ON LACTOBACILLUS ACIDOPHILUS CULTURES*

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Some effects of storage on *Lactobacillus acidophilus* cultures have already been reported (1). It was concluded that *L. acidophilus* milk cultures could be stored at either refrigerator or room temperatures for several days without any considerable destruction of the organisms, provided that the original acidity was not too high. The results then obtained were secured with milk cultures of original acidities from 0.50 to 0.80 per cent, expressed as lactic acid.

The data here presented were obtained upon milk cultures of *L. acidophilus* that were high in acidity when stored.

PROCEDURE

In addition to strain A4 and A15 previously reported upon for low acidities,[1] three additional strains were used, A2, A5 and A10. These strains were all stock cultures.

Flasks containing 100 cc. of skimmed milk were sterilized by heating at 15 pounds pressure for twenty-five minutes. After cooling, they were inoculated with 1 per cent of a milk culture of the respective strains and incubated at 37°C. A fresh flask for each strain was inoculated at twelve-hour intervals for a period of eighty-four hours. This resulted in a total of 8 flasks for each strain, each having a twelve-hour-longer incubation period than the next one in the series.

* Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 174, College of Agriculture and Experiment Station, State College of Washington. Received for publication September 5, 1930.

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[1] Black, L. A. 1931 Viability of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* cultures stored at various temperatures. Jour. Dairy Sci., xiv, 59–72.

The flasks were allowed to remain in the incubator until after the most recently inoculated flask had coagulated. Since the rate of coagulation varied somewhat among the strains, this resulted in a longer period of incubation in some cases. All flasks of each strain were removed from the incubator at the same time, thus giving cultures of varying ages. This also resulted in corresponding variations in acidity.

The acidity of each culture was determined by titration of a 10-cc. sample. A sample was removed for plating after which each culture was divided, one portion was left at room temperature and the other was placed in the refrigerator. The room temperature varied from 20° to 24°C. and the refrigerator temperature was approximately 9°C. Three cultures were left at these temperatures for five days, two for six days and 1 culture for nine days, when the acidity was again determined and plates were poured.

Bacto-dehydrated whey agar was used for all plating. Duplicate plates were poured and incubated at 37°C. for four or five days. All strains were incubated in an atmosphere containing 5 per cent carbon dioxide, except the first experiment on strain 4 which was in an atmosphere containing 10 per cent carbon dioxide. These percentages of carbon dioxide had been found by previous experiment to favor the growth of the strains used. Water in a flask was displaced by carbon dioxide from a tank, and then by replacing measured amounts of water in the flask, the carbon dioxide was forced into the petri plate containers which, in these experiments, were vertical autoclaves. In this way carbon dioxide, to the extent of 5 or 10 per cent of the volume of the container could readily be measured.

No circulation of the carbon dioxide was attempted with strains 4, 2 and 5. During the incubation of the strains 4a, 10 and 15, the atmosphere surrounding the plates was kept in slow circulation. This was accomplished by pumping the air from the bottom of the container and returning it at the top of the petri plate container, using a closed circulation system.

RESULTS

The results obtained are presented in table 1.

TABLE 1

Viability of L. acidophilus cultures at various acidities

CULTURE	INCUBATED	ORIGINAL		STORED	REFRIGERATOR		ROOM	
		Acidity	Count		Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*days*	*per cent*	*millions*	*per cent*	*millions*
4–1	5	1.65	56.0*	9	1.82	—	1.91	—
4–2	4.5	1.58	192.5		1.49	31.0	1.86	—
4–3	4	1.52	103.0		1.70	—	1.92	—
4–4	3.5	1.40	187.0*		1.47	63.5	1.76	—
4–5	3	1.14	186.0*		1.35	256.5	1.67	107.0*
4–6	2.5	0.87	92.0*		1.20	—	—	—
4–7	2	0.73	106.0		1.04	282.0*	1.33	196.0
4–8	1.5	0.44	89.0		—	—	—	—
2–1	7	1.88	33.5	6	2.12	—	2.16	—
2–2	6.5	1.79	63.0		2.14	—	2.11	—
2–3	6	1.72	117.0*		1.91	7.9	2.08	20.5*
2–4	5.5	1.36	40.0		1.93	—	2.09	—
2–5	5	1.40	97.0		1.68	24.1	1.93	20.1
2–6	4.5	1.23	42.5		1.53	81.0	1.79	61.0*
2–7	4	0.89	189.0		1.13	—	—	—
2–8	3.5	0.55	82.5		0.91	67.0	1.32	39.5
5–1	7	1.62	6.0	6	1.86	—	2.34	—
5–2	6.5	1.58	35.0		1.85	—	1.83	—
5–3	6	1.50	87.0		1.69	19.6	1.69	—
5–4	5.5	1.35	113.5		1.68	31.9	1.71	22.1
5–5	5	1.31	93.0		1.49	30.5	1.62	—
5–6	4.5	1.22	81.0		1.39	52.7	1.58	10.1*
5–7	4	0.95	219.0*		1.31	120.0	1.36	—
5–8	3.5	0.71	221.0		0.99	194.0	1.07	86.0
4–a1	5	1.89	36.0	5	1.78	118.5	1.92	3.0
4–a2	4.5	1.75	126.5		1.76	88.0	1.78	10.0
4–a3	4	1.73	352.5		1.66	130.0*	1.74	87.5
4–a4	3.5	1.59	173.0		—	313.5	1.64	130.0
4–a5	3	1.55	319.5		1.45	265.0	1.62	165.0
4–a6	2.5	1.15	361.5		1.39	186.0*	1.49	173.0
4–a7	2	1.00	542.0*		1.39	410.0	1.49	216.0
4–a8	1.5	0.92	144.0*		1.15	363.0	1.27	197.0

* Result of one plate.
— No count, usually moulds.

TABLE 1—*Concluded*

CULTURE	INCUBATED	ORIGINAL		STORED	REFRIGERATOR		ROOM	
		Acidity	Count		Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*days*	*per cent*	*millions*	*per cent*	*millions*
10–1	4.5	1.67	11.2	5	1.67	6.3	1.73	6.7
10–2	4	1.62	15.7		1.61	11.5	1.72	7.4
10–3	3.5	1.52	1.3		1.46	22.5	1.66	21.1
10–4	3	1.35	51.0		1.26	3.5	1.64	11.4
10–5	2.5	1.30	44.5		1.17	3.8	1.27	20.5
10–6	2	1.19	392.5		0.96	13.3	1.04	20.5
10–7	1.5	0.68	531.5		0.79	15.5	0.87	26.6
10–8	1	0.45	302.0		0.55	16.0	0.74	23.9
15–1	5	1.87	2010.0*	5	1.89	2250.0	1.90	1950.0
15–2	4.5	1.88	1750.0		1.82	2110.0	1.93	2130.0
15–3	4	1.63	2200.0		1.80	2800.0	1.89	2350.0*
15–4	3.5	1.64	2260.0		1.71	1960.0*	1.77	2190.0*
15–5	3	1.43	2330.0		1.38	2340.0	1.78	3530.0
15–6	2.5	1.24	2735.0		1.28	2515.0	1.47	2465.0
15–7	2	0.93	2090.0		1.07	1715.0	1.31	2500.0
15–8	1.5	0.85	1900.0		1.09	780.0	1.24	2160.0

DISCUSSION

The results presented in table 1 indicate that the highest count of organisms was obtained at the lower or moderate acidities and that the greatest destruction of organisms occurred in the more acid cultures. The original cultures will be considered first in the discussion to follow; that is those cultures that were not stored. They will be discussed in the order given in table 1.

It is believed that the factor of age can be largely eliminated in the consideration of the results presented in table 1. In culture 10, however, it seems that age or some other factor operated in drastically reducing the number of organisms in all samples upon storage. It is not likely that acidity could here be the predominating factor since at the low acidity of 0.55 per cent the same severe reduction was obtained as occurred at the higher acidities. This factor might also account for the more rapid reduction occurring with this culture, strain 10, in the higher acidities of the original series.

The highest counts in strain 4 were obtained at acidities of 1.14, 1.40 and 1.58 per cent. Samples were not obtained between 0.87 and 1.14 per cent acidity. Judging from the refrigerator storage samples of this series, and from the second set of results upon this strain, given in table 1, it seems probable that the greatest number of organisms was reached between the acidities of 0.87 and 1.14 per cent. Destruction of the organisms in strain 4 had probably started at 1.14 per cent acidity. The number of viable organisms then remained rather constant through 1.58 per cent with a greater decrease at 1.65 per cent acidity.

The highest count in strain 2 was obtained at an acidity of 0.89 per cent. The next highest acidity in the series was 1.23 per cent. It seems likely that the greatest number was reached between these acidities. This would then correspond closely with the results obtained with strain 4. Destruction of the organisms had probably occurred at 1.23 per cent acidity and the number surviving then remained more or less constant through the remainder of the series.

The highest counts in strain 5 were obtained at acidities of 0.71 and 0.95 per cent. This is somewhat lower than in the case of the strains already mentioned. Destruction of the organisms had started at 1.22 per cent acidity. The number of organisms remained about the same through 1.5 per cent but increased destruction took place at 1.58 and still more at 1.62 per cent acidity.

In the second series of experiments on strain 4, the largest number of organisms were obtained at 1.00 per cent acidity. This corresponds to the conclusion reached in the first series as judged by the refrigerator storage samples. Destruction in the second series had begun at 1.15 per cent acidity. The number of organisms remained somewhat the same through acidities to 1.73, decreasing at 1.75 and decreasing greatly at 1.89 per cent acidity.

The largest number of organisms in the series of strain 10, were obtained at 0.68 per cent acidity. This resembles strain 5, which had the highest count at an acidity lower than strains 2 and 4. Destruction of organisms in strain 10 had commenced at 1.19 per cent acidity. A great destruction had again occurred at 1.30

per cent acidity, the number of organisms then remaining more or less constant until further decreases took place at acidities of 1.5 per cent and above. As mentioned above, some of this destruction at 1.3 per cent acidity may be due to the greater effect of age or other factors upon this strain.

Strain 15 has always differed from some dozen other strains of *L. acidophilus* that have been examined. This strain was used because it was received from a laboratory specializing in acidophilus cultures, and was stated to be recently isolated from the human intestinal tract. For about two years this culture has given some ten times as high a count as other acidophilus strains that have been used.

Strain 15 also seems to be more resistant to acid than the other strains used. An earlier experiment on this strain, similar to the series reported in table 1, gave essentially the same results. This earlier series could not be counted inasmuch as a one million dilution was the highest used. At the end of the experiment, however, the plates were all placed in order and a rough comparison made. Destruction of organisms could not be detected in the lower acidities until an acidity of approximately 1.5 per cent was reached. At the higher acidities somewhat greater reductions occurred than in the present series of experiments. It can be concluded, therefore, that strain 15 is more resistant to the acid formed than are the other cultures used in the present experiments.

The largest number of organisms in strain 15 was secured at 1.24 per cent acidity in the original set of cultures. This is at a higher acidity than was found for strains 2 and 4 and considerably higher than strains 5 and 10. That it is really higher is borne out by the highest counts in the stored samples, which were obtained at still higher acidities. Destruction of the organisms in the original set had started at 1.43 per cent acidity. Further decreases in numbers were indicated after 1.88 and 1.87 per cent acidity.

The results indicate then, that the highest number of organisms reached with regard to acidity was as follows: strain 4, 0.87 to 1.14; strain 2, 0.89 to 1.23; strain 5, 0.71 to 0.95; strain 4, 1.00;

strain 10, 0.68, and strain 15, 1.24 per cent. Destruction had apparently occurred at the following acidities: strain 4, 1.14; strain 2, 1.23; strain 5, 1.22; strain 4, 1.15; strain 10, 1.19, and strain 15, 1.43 per cent. A still more pronounced destruction of organisms had taken place in each of the strains except 2 at the following acidities: strain 4, 1.65; strain 5, 1.58 and 1.62; strain 4, 1.75 and 1.89; strain 10, 1.3 and 1.52, and strain 15, 1.87 per cent.

In the data presented strains 2 and 4 gave the highest plate counts at approximately 1.00 per cent acidity, strains 5 and 10 at less than 1.00 per cent and strain 15 at an acidity above 1.00 per cent. A decrease in the number of organisms seemed to have occurred when 1.2 per cent acidity had been reached in all strains except 15. There was considerable variation in the higher acidities at which still greater decreases in viable organisms were evident.

When we consider the highest numbers secured and the acidities at which a decrease occurs in the storage samples, we find that they correspond to the original unstored samples. The highest number of organisms, again, are found at the low acidities and the greatest destruction of organisms takes place in the more acid of the series.

In the cultures stored in the refrigerator the highest number in strain 4 was secured at 1.04 per cent acidity with a decrease at 1.35 per cent. With strain 2 counts were not secured between acidities of 0.91 to 1.53 per cent. It seems probable that destruction had begun at 1.53 per cent acidity or before with the highest number below this. In strain 5 the highest number was secured at 0.99 and decrease had taken place at 1.31 per cent acidity.

Continuing with the other refrigerated cultures, the second series of strain 4 exhibited the largest count at 1.39 per cent acidity. However, in another culture of the same acidity the number of organisms had already decreased. So it is probable that a decrease had already occurred at 1.39 per cent acidity and that the highest number had been reached before this.

As mentioned in the second paragraph of this discussion, some other factor seems to have operated in greatly reducing all storage

samples of strain 10. Strain 15, as mentioned above, seemed to be more resistant to the acidity produced than other strains. So in the refrigerated samples, a large number of organisms was secured at 1.28 per cent, followed by a decrease at 1.38 and 1.71 per cent. In this series the highest number was secured at an acidity of 1.80 per cent, followed by a decrease at 1.82 and 1.89 per cent acidity.

In the samples stored at room temperature, of strains 2, 4, 5 and the second series of strain 4, the highest count was in all cases secured in the sample corresponding to that giving the highest count in the refrigerated samples. In these cases there had been an increase in acidity of from 0.08 to 0.29 per cent in the samples stored at room temperature over that of the refrigerated samples.

In every case the highest count for the room storage samples was less than the count of the corresponding refrigerated sample. Decrease in numbers among a series of these room stored cultures proceeded like that of the refrigerated samples, except that in every instance the acidity for the corresponding culture was higher at room storage and in all but one case, culture 2–3, the count was correspondingly lower.

The general effect of storage in the refrigerator of the cultures at the various acidities was to decrease the numbers. In 11 of 38 samples, however, there was an increase in count above that of the original. Of these increases, 3 were at lower acidities, suggesting multiplication before chilling. In 4 other cases, it seems from the data that the counts were higher largely because the original count for that series was lower than would be expected from the other younger and older cultures of its series. The remaining 4 instances of an increased count were in strain 15.

The general effect of storage at the various acidities, then, is to decrease the numbers upon storage at 9°C. with a further reduction in numbers if stored at 24°C. In the first four strains presented in table 1, in only one case, culture 2-3, was there an increased number of organisms in room storage over refrigerator storage. In strain 10 the opposite effect prevails, but other factors must be involved there. With strain 15, in 5 instances a higher count was secured at room storage than with refrigerator

storage. In 15-8 and 15-7 it would seem that continued multiplication accounted for this increase at room storage. Whether this could explain the other three increases of the series is problematical.

The acidity in all cases but one was higher at room storage than at refrigerator storage. In this one instance the difference was 0.03 per cent. In all but 10 of the 48 refrigerated samples, the acidity had increased over that of the original. In these 10 instances, the variation was from 0.01 to 0.23 per cent, all but one being 0.11 or under, all averaging 0.095 per cent with 6 of the 10 samples below this average. It is probable that much of this difference might be due to difficulty in reaching the same end point on each titration, since the sterilized milk is slightly caramelized and so interferes with the color obtained at the endpoint.

In all but 2 of the 48 room storage samples, the acidity had increased over that of the original. These cases, 10-6 and 10-5, were 0.15 and 0.03 per cent lower and were the two samples that varied the most in the refrigerated samples. This might presumably have been due to too high a reading on the original set.

None of these cases of variations in acidity, which might have been due to error in recording the endpoint of the titration, concerned a series at which the highest number of organisms was secured. In only one case, 10-6, this might have resulted in a different acidity at which decrease was first evidenced. In the other instances of acidity variations the samples concerned were above that acidity in which decrease was first noticeable.

In connection with the experiments presented in table 1, the long period of incubation of *L. acidophilus* plates also pointed out conditions not noticeable in ordinary plate counts of shorter incubation periods. Molds did not appear within the two-day incubation period for standard plate counts, but with a five-day incubation of acidophilus organisms, plates sometimes could not be counted because of mold growth. These molds usually appeared as one or two large colonies covering most of the plate. The molds seemed to enter the plates after the plates were inverted and placed in the incubator. After growing down between the inverted outer and inner dishes and then up inside the inner

plate to the media, the mold spread from the edge of the plate, covering large areas. In certain experiments in closed containers, it seemed that a very few mold spores could thus grow and spread from one plate to another, infecting practically every plate within five days incubation.

From the results given, it seems that the method of incubating until different acidities are reached, plating at these acidities and drawing conclusions from the counts obtained, gives evidence regarding the effect of storage at the various acidities. The largest counts in the original set of samples were obtained at acidities lower than was the case with the stored samples, yet in general the samples giving highest counts upon storage was identical with those giving the highest count in the original. This would indicate that the increase in acidity before chilling was completed was not particularly harmful if the cultures were removed from the incubator when the maximum number of organisms was reached. If the cultures were to be stored at room temperature, it is reasonable to suppose that at some acidity under that giving the maximum number in the original there would be slightly more multiplication at room temperature and so yield more organisms after storage than would result from storage of the sample giving the maximum number before storage.

It is suggested that results might be more readily obtained by using different amounts of inoculation and plating more than once from the same sample. This should enable one to secure samples within a closer range of acidities than was possible in the experiments here presented.

SUMMARY

1. The strains of *L. acidophilus* used in the experiments reported upon gave the highest plate counts at acidities of approximately 1.00 per cent.

2. There was some variation among strains, some yielding the maximum number of organisms at less than 1.00 per cent and one strain at an acidity considerably greater than 1.00 per cent.

3. A decrease in the number of organisms had taken place at an acidity of approximately 1.2 per cent in all but one strain.

The strains examined seemed to differ slightly with respect to the acidity at which destruction became apparent.

4. At higher acidities of approximately 1.5, 1.6, 1.7 and 1.8 per cent, a still greater destruction of organisms took place. The acidity at which this greater destruction occurred varied with the strain.

5. The results indicated that the particular sample of each strain that gave the highest count before storage, usually gave the highest count of the series after storage at 9° or 24°C.

6. Refrigerator storage (9°C.) of typical strains of *L. acidophilus* resulted in a smaller loss in the numbers of organisms than did storage at 20° to 24°C., in both high and low acidities.

7. Two strains of *L. acidophilus* not previously reported upon also survived either refrigerator or room temperature storage for several days without any great destruction of organisms, provided that the original acidity was low.

8. It is desirable to determine the acidity at which the maximum number of organisms is secured for each strain and under the conditions that obtain.

9. The results obtained indicated that the acidity at which the maximum number of organisms was secured during incubation of a culture was an index as to the acidity at which to place cultures in refrigerator storage.

THE NUMBER OF DAUGHTERS NECESSARY TO PROVE A SIRE*

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Increased appreciation of the progeny test as a means of rating the real breeding worth of an animal is a noteworthy development in modern animal breeding. The idea of a progeny test is not a new one. The best breeders have always relied upon it, although in a somewhat indefinite and approximate way. Why a progeny test is more accurate and useful than a rating of the individual by what it appears to be itself, or by what its pedigree shows that its ancestors were, began to be evident soon after the rediscovery of Mendelism but only in the last ten years have adequate methods been available for combining genetics and statistical procedure to study the usefulness of the progeny test under varying conditions.

It has always been known in a general way that a progeny test increases in reliability as the number of offspring included in that progeny increases. That this increase in reliability might be expressed very definitely by a fairly simple formula was first suggested by critical examination of Illinois Station Bulletin 270, which appeared in 1925. This bulletin was an empirical study of bulls having 15 or more daughters entered in the Register of Merit of the American Jersey Cattle Club. Figure 1 is modelled after figure A of that bulletin with three smooth lines showing the theoretical results if the simple correlation between the records of half sisters had been 0.25, 0.30, or 0.36 and if the number of bulls tested had been large enough to equalize all errors of random sampling.

The study in that bulletin was limited to the correlation between a small number of daughters and some arbitrarily chosen larger number. It is possible to go a step further and derive a

* Received for publication September 12, 1930.

general formula[1] for the correlation between the average record of the daughters and the genotype of the sire for factors affecting milk production.

In deriving these general formulas allowance should be made for the fact that the primary correlation between the records of half sisters is in itself a compound figure, made up partly of the fact that half sisters tend to have received somewhat similar inheritance from their sire, and partly of the fact that half sisters are usually reared in the same herd and are therefore to some extent subject to common conditions of environment, feeding and system of management. Also the dams of the half sisters may in general have an appreciable relationship to each other. This compound nature of the simple correlation between the records of half sisters is shown in figure 2, which is drawn according to Dr. Wright's path coefficient method of analyzing correlation coefficients into their component parts. If we let s represent the path coefficient from the sire's genotype to the daughter's record and if we let e represent the path coefficient from the herd management or common environment (including also the average relationship of the dams to each other) to the daughter's record then it is evident that the primary observed correlation between the records of half sisters equals $e^2 + s^2$. (This formula does not allow for re-

[1] The mathematical and genetic principles underlying such formulas as this have been stated by Dr. Sewall Wright ("Correlation and Causation," Jour. Agr. Research, xx, 557–585, and "The Biometric Relations Between Parent and Offspring," Genetics, vi, 111–123). A correlation coefficient measures the tendency of two things to vary together and thus to be predictable from each other. In this case the correlation between the genotype of the sire and the average record of his daughters measures how closely the real breeding worth of the sire for milk and fat production can be predicted from the records of the daughters. A correlation of 1.0 would mean that the bull's breeding worth is perfectly predictable, with no room at all for error. A correlation of 0.9 would mean that predicted values and actual values will be nearly alike and most of the errors which do occur will be small ones. In general a correlation of 0.5 expresses the resemblance between one parent and one offspring while a correlation of 0.7 expresses the general tendency of one offspring to be like the average of both its parents where (1) neither the parents nor the offspring are highly inbred or distinctly crossbred, (2) the character being studied is not markedly influenced by the variations in environment to which that population is exposed, and (3) where few if any of the genetic factors, governing the characteristic being studied, are completely dominant or complementary in their action.

lationship between sire and dam, but such relationship is negligible in most material on account of the widespread avoidance of all systems of mating animals very closely related to each other.) Besides the number of daughters included in the average, the formulas in figure 2 require an evaluation of s and of e before they can be solved in numerical terms. The value of s would be 0.50

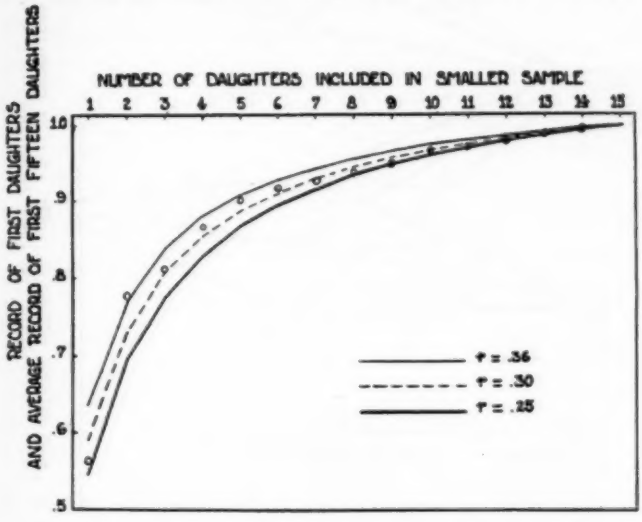
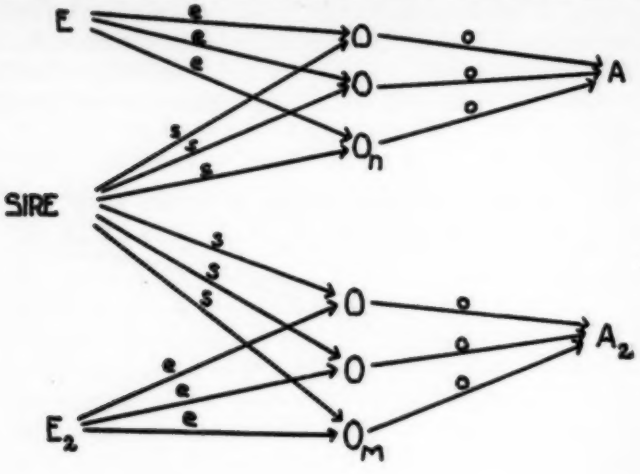

NUMBER OF DAUGHTERS INCLUDED IN SMALLER SAMPLE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
RECORD OF FIRST DAUGHTERS AND AVERAGE RECORD OF FIRST FIFTEEN DAUGHTERS
1.0
.9
.8
.7
.6
.5
r = .36
r = .30
r = .25

FIG. 1. THEORETICAL AND OBSERVED CORRELATIONS BETWEEN THE AVERAGE RECORD OF THE FIRST FEW DAUGHTERS AND THE AVERAGE RECORD OF THE FIRST FIFTEEN DAUGHTERS

The circles show the values actually found when the original data on which Illinois Bulletin No. 270 was based were recalculated, grouped in smaller class intervals than used in that bulletin. Dr. Davidson's kindness in supplying the original data made possible a more adequate test of the general formula theoretically derived.

in a random-bred population where there was no dominance and where milk and fat production were entirely determined by heredity. If dominance is at all widespread in the factors affecting milk and fat production this value would be slightly lowered. We know that milk and fat production are not entirely determined by heredity and this also would lower the value of s. On the

other hand whatever has been accomplished through selective breeding of like to like among the ancestors of the sire would tend



$$R^2_{A\cdot Sire} = \frac{ns^2}{1+(n-1)(e^2+s^2)}$$

$$R^2_{A\cdot A_2} = \frac{nms^4}{[1+(n-1)(e^2+s^2)][1+(m-1)(e^2+s^2)]}$$

FIG. 2. DIAGRAM SHOWING THE COMPOUND NATURE OF THE CORRELATION BETWEEN A SIRE'S GENOTYPE AND THE AVERAGE RECORD (A) OF HIS DAUGHTERS

The even more compound nature of the correlation between the average record (A) of his daughters in one herd and the average record (A_2) of his daughters in another herd is also shown. O represents the record of one daughter and E represents the herd management and environment which affect all the cows in one herd but may be very different (E_2) in other herds.

to raise the value of s. It does not seem possible at present to evaluate s exactly. Its most probable value seems, taking everything into account, to be not far from 0.40 or perhaps a little

above that in studies restricted to official records. In table 1, s has been figured at four different values; namely, 0.30, 0.40, 0.50, and 0.60, although it is doubtful that either of the extreme values is often reached in actual practice. The value of e is even less definitely determined but would be larger in such a group as D. H. I. A. records than it would in the advanced registry records

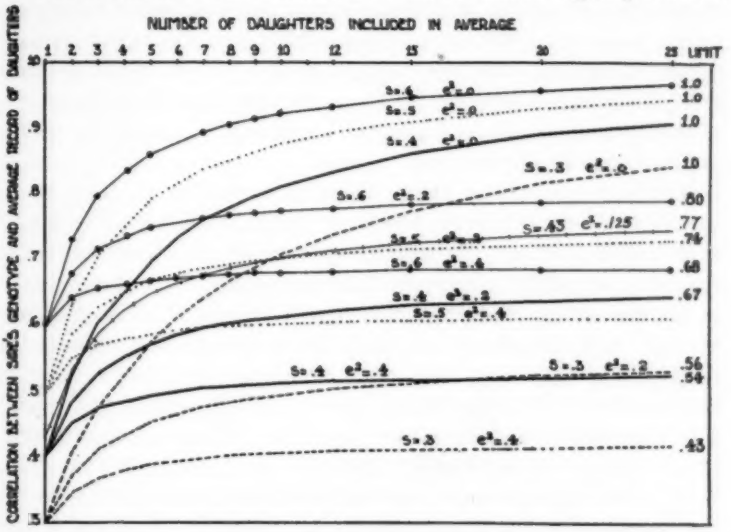


FIG. 3. THE RELATION BETWEEN THE NUMBER OF DAUGHTERS IN THE PROGENY AND THE RELIABILITY OF THE PROGENY TEST

Expected correlation between sire's genotype and the average record of various numbers of daughters at selected values of s and e^2.

since the cows on advanced registry tests are more nearly given every chance to show their producing ability than are cows under D. H. I. A. conditions. On the basis of all analyses yet made of advanced registry records it seems likely that the most probable value of e^2 in advanced registry data is not far from 0.15 and that it can rarely if ever be as large as 0.30. In the calculations, values of 0.00, 0.10, 0.20, 0.30, and 0.40 have been assumed for e^2. Dr. Wright in an unpublished analysis finds that the results of

Gowen's analysis of Holstein-Friesian Advanced Registry records are consistent with values of about 0.435 for s, 0.125 for e^2 (not including the relationship of the dams to each other), and 0.20 for the average relationship of the dams to each other.

Figure 3 shows the expected correlation between the genotype of the sire and the average of his daughters' records for some of these selected values of e^2 and s where the number of daughters varies from 1 to 10 and upward. These curves differ greatly among themselves but they have certain characteristics in common, most noteworthy of which is the fact that all of them rise abruptly as the number of daughters increases but that the curves tend very soon to flatten out.

Another noteworthy point about these curves is that all of them have limiting values less than 1.0 except where e^2 is 0 and even then the value, 1.0, is only approached as a limit when the number of daughters becomes infinitely large. In practical language this means that we cannot expect the progeny test to be absolutely and completely accurate even though the number of daughters is very large and every precaution is taken to control and standardize the conditions under which the records are made. When the number of daughters is small, s is in general more important than e in determining the reliability of the progeny test. If differences in herd environment and management are great in the herd or section of the breed under consideration and if the records are not corrected accurately for those differences, then the ultimate limit of the progeny test is low and for most practical purposes it increases in reliability only a little after the first half dozen or so daughters have made records. On the other hand if the effects of differences in common environment are relatively slight (or if accurate corrections are made for those differences) and if the variations in records are largely due to genetic differences between the heifers and to uncontrollable environmental forces operating differently, even on heifers in the same herd, then the reliability of the progeny test continues to increase noticeably even beyond the point where one has as many as ten or a dozen daughters already tested.

The more carefully environmental conditions are controlled

and made uniform for all of the daughters which are to be tested, just so much more is the value of e lowered in making comparisons with other bulls whose daughters have been tested under similarly controlled conditions. The use of accurate correction factors for known and measured differences in herd environment serves the same purpose as actual control of the physical and biological environment, but of course can not entirely eliminate e because correction factors are only approximate, even when most carefully derived. The value of e is also slightly raised by making environmental conditions uniform throughout the herds or section of the breed under consideration (or by making proper correction for such conditions when they cannot be made uniform), since a larger proportion of the observed differences thus are determined by heredity than if conditions were less well controlled. Thus careful control of environmental conditions throughout the herd or portion of the breed considered operates to make the progeny test more reliable. This difference in reliability can become very great as may be seen by comparing the extreme outside curves in figure 3.

Such careful control of (or allowance for) environmental conditions can not entirely eliminate e^2 but may hold it down to a low level, perhaps to less than 0.10. The value of s can go above 0.5 only where a very unusual amount of inbreeding (homozygosity) is involved. Even for s to have a value of 0.6 the sire would need to be 44 per cent more inbred (homozygous) than his daughters out of unrelated or slightly related cows, or if not inbred himself he must be mated to cows more closely related to him than half sisters. That makes no allowance for the part played by dominance or by uncontrolled environmental influences in determining the daughters' records. Such a part, even though small, would necessitate still more intense inbreeding if the value of s is to approach 0.6. From these considerations it is evident that a value for s as high as 0.6 is almost unattainable with the systems of breeding prevalent in dairy cattle today. The curve for $s = 0.5$, $e^2 = 0.0$ in figure 3 probably comes close to the maximum reliability of the progeny test in any very large group of dairy herds today, even with environmental conditions as perfectly controlled

or corrected for as is possible. Even if the value of s did go a little above 0.5 in certain special groups, yet e^2 would still have at least a slight value. This would result in a curve starting a little higher and rising a little steeper but flattening out sooner to a lower limit than the curve shown for $s = 0.5$, $e^2 = 0.0$.

The discussion this far has been on the assumption that the daughters' records would be used without taking into account the records of the dams of those daughters. Where the dams also have records, it seems reasonable to suppose that accuracy would be much increased by taking those records also into account. Actually this gain, though real, seems to be comparatively small. Thus, under the conditions of random mating, complete determination by heredity, and no dominance, the value of s rises from 0.50 where the dam's record is not considered at all only to 0.58 where the dam's record is compared with the daughter's record, each being weighted in the most ideal manner, i.e., with the daughter's record given twice as much weight as her dam's record. In so far as the daughters and dams are tested in the same herds the same year, the use of daughter-dam comparisons will also tend to give e^2 a lower value than if no use were made of the dams' records. From these theoretical considerations it seems that the use of daughter-dam comparisons should render the progeny test more reliable but that this effect will be less than is generally believed. It is hardly possible that s could thus be raised to the extreme value (0.6) shown in figure 3 but it might conceivably be raised above 0.5 where all environmental circumstances were controlled (or corrected for) in the most exact manner possible. e^2 could not be eliminated entirely but might possibly be held below 0.2 or perhaps even below 0.1 with excellent control (physical or statistical).

The comparison between pedigree promise and progeny test is especially interesting. For characters completely determined by heredity without dominance and in a random-bred population, the correlation between the individual and the most perfect system of averaging its ancestors is limited to about 0.71. Under the same conditions the correlation between the individual and the average of its offspring becomes this high when there are as many as three

offspring included in the average. Under these same conditions when more than three individuals are available the progeny test becomes more reliable than pedigree promise. No one seems yet to have worked out the consequences of variations in s and e on the general reliability of the pedigree as an indication of what the individual will be. It is obvious that e will usually be smaller in a pedigree than in a progeny test since rarely will all of the ancestors have been reared under environmental conditions which were all good or all poor. On the other hand in dairy cattle there is no direct measure of productiveness in the males and this limits our ability to estimate the productiveness of an individual by a study of its pedigree. It seems likely therefore that the correlation between an animal's productiveness and what one might estimate that productiveness to be from a careful study of the pedigree, even when all female ancestors had been tested, would probably very rarely go over 0.65 and perhaps might not even be as high as 0.55 under ordinary conditions.

How then does the progeny test compare with pedigree promise as a basis for estimating an individual's real genotype for milk and fat production? Obviously there is no single answer which will fit all conditions alike. In general when there are as many as four to six offspring included in the progeny test, that test will be about as accurate as an estimate based on even a very complete pedigree. A progeny test based upon larger numbers than this will often be distinctly more reliable than all the information to be gained by careful and long-continued study of the pedigree. However there are sure to arise occasions when sires with good genotypes are used in herds where their daughters are given poor care while other bulls will be used in herds where the daughters are given excellent care and management. Under those circumstances the progeny test by itself may not be as reliable a basis for estimating that bull's heredity for milk and fat production as his pedigree would have been. On the other hand where all environmental conditions are made as uniform as possible for all of the daughters of the different bulls which are being tested, the progeny test can approach perfection as an indicator of the bull's breeding worth, while pedigree estimates must necessarily be

TABLE 1

Correlation between sire's genotype and the average record of his daughters

s	e^2	NUMBER OF DAUGHTERS INCLUDED IN AVERAGE																
		1	2	3	4	5	6	7	8	9	10	12	15	20	25	50	100	∞
0.60	0.0	0.60	0.728	0.792	0.832	0.859	0.878	0.893	0.905	0.914	0.921	0.933	0.946	0.958	0.966	0.983	0.991	1.000
	0.1	0.60	0.702	0.750	0.778	0.796	0.809	0.819	0.826	0.832	0.837	0.844	0.852	0.860	0.865	0.874	0.880	0.885
	0.2	0.60	0.679	0.714	0.733	0.745	0.754	0.760	0.765	0.769	0.772	0.777	0.782	0.786	0.789	0.796	0.799	0.802
	0.3	0.60	0.659	0.682	0.695	0.703	0.709	0.713	0.716	0.718	0.720	0.723	0.726	0.729	0.731	0.735	0.737	0.739
	0.4	0.60	0.640	0.655	0.663	0.667	0.671	0.673	0.675	0.676	0.678	0.679	0.681	0.683	0.684	0.686	0.687	0.688
0.50	0.0	0.50	0.632	0.707	0.756	0.791	0.816	0.837	0.853	0.866	0.877	0.894	0.913	0.933	0.945	0.971	0.985	1.000
	0.1	0.50	0.609	0.664	0.698	0.722	0.739	0.751	0.761	0.769	0.776	0.786	0.797	0.808	0.815	0.830	0.837	0.845
	0.2	0.50	0.587	0.628	0.652	0.668	0.679	0.688	0.694	0.699	0.704	0.710	0.717	0.724	0.728	0.736	0.741	0.745
	0.3	0.50	0.568	0.598	0.614	0.625	0.632	0.638	0.642	0.645	0.648	0.652	0.657	0.661	0.663	0.669	0.671	0.674
	0.4	0.50	0.550	0.571	0.582	0.589	0.594	0.598	0.600	0.602	0.604	0.607	0.609	0.612	0.614	0.617	0.619	0.620
0.40	0.0	0.40	0.525	0.603	0.658	0.698	0.730	0.756	0.777	0.795	0.810	0.834	0.861	0.890	0.909	0.951	0.975	1.000
	0.1	0.40	0.504	0.562	0.603	0.626	0.646	0.661	0.674	0.684	0.692	0.705	0.719	0.734	0.743	0.763	0.774	0.784
	0.2	0.40	0.485	0.528	0.555	0.573	0.586	0.595	0.603	0.609	0.614	0.622	0.630	0.639	0.644	0.655	0.661	0.667
	0.3	0.40	0.468	0.500	0.519	0.531	0.539	0.546	0.551	0.555	0.558	0.563	0.568	0.573	0.576	0.583	0.586	0.590
	0.4	0.40	0.453	0.476	0.489	0.497	0.503	0.507	0.510	0.513	0.515	0.518	0.521	0.524	0.526	0.530	0.532	0.535
0.30	0.0	0.30	0.406	0.478	0.532	0.575	0.610	0.640	0.665	0.686	0.705	0.737	0.773	0.815	0.844	0.912	0.953	1.000
	0.1	0.30	0.389	0.442	0.479	0.506	0.526	0.543	0.556	0.567	0.576	0.591	0.607	0.625	0.636	0.661	0.674	0.688
	0.2	0.30	0.374	0.413	0.439	0.456	0.469	0.480	0.487	0.494	0.499	0.508	0.517	0.526	0.532	0.544	0.550	0.557
	0.3	0.30	0.360	0.389	0.407	0.419	0.428	0.434	0.439	0.443	0.447	0.452	0.457	0.463	0.466	0.473	0.477	0.480
	0.4	0.30	0.348	0.369	0.382	0.390	0.396	0.400	0.403	0.406	0.408	0.411	0.414	0.418	0.420	0.424	0.426	0.429

limited to a value of about 0.7 or less except in pedigrees where there has been a very significant amount of close breeding. There is a legitimate place for the use of both pedigree and progeny test in rating a bull's real breeding worth and also room for attention to the bull's individuality. As the progeny test becomes more thorough and reliable we should of course give it relatively more attention in making our estimates. This means that pedigree and the bull's individuality will receive relatively less attention but we cannot now foresee any logical reason for neglecting them altogether, even in the most adequate of progeny tests possible.

What then is the number of daughters which must be tested before we can have an adequate progeny test of the sire? Obviously there is no number below which we must say the test is inadequate and above which we can say that the test is certainly correct. Our reliance in the progeny test should increase as the number of daughters increases, but at an ever-decreasing rate. At low values of s and e we may gain a very noticeable increase in the reliability of the progeny test by increasing the number of daughters even past eight or ten. At the other extreme with high values of both e and s there is little accuracy to be gained by increasing the number of daughters even past three or four. For what appear to be the most probable values for s and e only a little increase in accuracy is to be gained by including more than about five to eight daughters in the progeny test although of course one would want to base his estimate upon all that are available no matter how many that may be.

If a definite number must be adopted in order formally to define what a "proved sire" is, perhaps the number five, adopted by the Bureau of Dairy Industry, is as practical as any other. Certainly it is distinctly more accurate than a progeny test based on three or four except when the value of e is quite high. On the other hand the increase in accuracy which would be gained by requiring more than five tested daughters in order to "prove" a sire would be large only when the values of e and s are low. From the principles underlying the behavior of the progeny test, it must be quite obvious that we will occasionally encounter cases where a sire "proved" to be good in one herd will with equal cer-

tainty "prove" to be bad in another. Some sires will be "proved" to be poor ones merely because of chance variations or because they were used in a herd where the care and management given their daughters were not adequate.

The progeny test is rightly esteemed as one of the most valuable weapons which the breeder has at his command and everyone interested in the breeding of better livestock will do everything possible to promote its wider use, but we will save ourselves from some severe and perplexing disappointments if we keep in mind the principles on which the reliability of the test is based and thus know how much confidence to place in it.

THE INFLUENCE OF SWEETENED, FROZEN CREAM ON THE DEVELOPMENT OF SWELL IN ICE CREAM*

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The storage of milk fat for use in ice cream is a common procedure. It is usually held in frozen storage in the form of unsalted butter, butter oil, or low-acid cream. Within the past few years it has become common practice in the ice cream industry to add from 10 to 40 per cent of sugar to low-acid cream before it is placed in storage. The sweetened or non-sweetened cream is stored in tin cans, milk cans, and, occasionally, wooden barrels.

The use of non-sweetened frozen cream in ice cream causes certain changes in the physical characteristics and freezing properties of ice cream mix. According to Price and Whitaker (1), the basic viscosity of ice cream mix is increased and the whipping properties are decreased by the use of frozen cream.

The changes, which are caused in ice cream mix by the use of frozen cream, are almost identical to the changes which are caused by the use of butter instead of fresh cream. Whitaker (2) believes that the difference in the lecithin content of ice cream mix which contains cream and one which contains butter is responsible for these changes. He points out that replacing the lecithin, which was removed by the churning of the cream, by using buttermilk or egg yolk, both of which contain lecithin, restores the whipping properties of the butter mix.

Urbain and Miller (3) investigated the effect of freezing on egg white and egg yolk and found that the only significant change was the separation of lecithin from the egg yolk. They showed that the addition of sucrose, dextrose and levulose in concentrations of 1 part sugar to 10 parts of egg yolk prevented this separation, and that the sucrose was less effective, in equal concentrations, due to

* Received for publication September 15, 1930. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

the difference in its influence on the osmotic pressure of the mixture of egg yolk and sugar. This explanation is also supported by the evidence of a patent (4) which involves the use of salt for the preservation in frozen storage of the emulsifying properties of egg yolks for the manufacture of mayonnaise.

Since the emulsifying properties of egg yolk lecithin are altered by freezing and thawing, it is possible that the colloidal properties of cream may be altered by similar treatment. Urbain and Miller showed that mixtures of sugar and egg yolks prevented the separation of lecithin during freezing. The addition of sugar to cream, which is to be frozen and stored for subsequent use in ice cream, may also prevent those changes in the colloidal system of the cream that cause the decrease in the development of overrun of the mix.

A series of experiments were planned to test the effect of the addition of sugar to cream which was subsequently frozen, held, thawed, and then used to make ice cream.

THE EFFECT ON THE DEVELOPMENT OF SWELL OF USING CREAM, FROZEN FOR 48 HOURS, WITH AND WITHOUT SUGAR

A quantity of fresh pasteurized cream, containing 45 per cent fat, was divided into 4 lots. The first portion was stored at approximately 35°F. The second was frozen at −15°F. Sucrose was added to the third portion at the rate of 1 part of sugar to 10 parts of cream, by weight. Invert syrup, which was made by inverting sucrose with citric acid, according to the method described by Jordan (5), was added to the fourth portion of the cream at the rate of 1.33 parts of syrup to 10 parts of cream by weight. This amount of syrup is equivalent to 1 part of sugar to 10 parts of cream. The third and fourth lots were also frozen at −15°F. All four lots were held in well-tinned ice cream cans.

The storage period lasted for forty-eight hours and then the frozen lots were placed in cold water and allowed to remain undisturbed until all ice disappeared.

The effects of the storage treatments on the appearance of the four lots of cream were clearly evident. The first lot, which had been held at 35°F., was unchanged. The second portion, which

contained no sugar, was granular due to the presence of small aggregates of milk fat, and minute particles of serum solids. The fat separated from the mix, in which this cream was used, in the form of oil during the pasteurizing period and was only incorporated by the homogenizing process. The third portion resembled the second but the aggregates of fat were smaller and less numerous and there was only a slight separation of milk fat during pasteurization. The appearance of the fourth portion was practically identical to that of the normal unfrozen cream. There

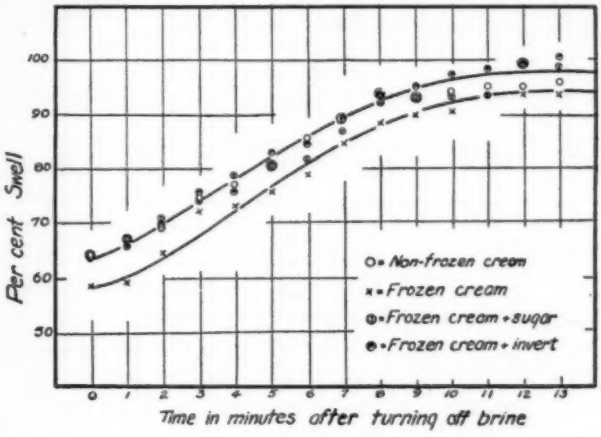

FIG. 1. THE INFLUENCE OF SWEETENED CREAM, FROZEN FOR 48 HOURS, ON THE DEVELOPMENT OF SWELL OF ICE CREAM

were no aggregates of fat visible to the naked eye and no fat separation.

Four mixes were made which differed from each other only in that they contained a different one of the four lots of cream. Each mix contained identical quantities of sugar and invert syrup, which together furnished 15 per cent of the weight of the mixes. Fat, skimmilk solids and gelatin, composed 12, 11 and 0.25 per cent, respectively of each mix. The skimmilk solids required, in excess of the quantity in the cream, were supplied by dry skimmilk. The mixes were pasteurized at 145°F. for thirty minutes,

homogenized at 3000 pounds pressure and then cooled to approximately 40°F. and aged at that temperature for sixteen to eighteen hours.

The procedure was repeated with four lots of cream during the period from April 11 to May 31, 1930. It is possible that the results might be different at other seasons of the year when the lecithin content of the milk might show the influence of other conditions.

The results of the different treatments, to which the four lots of cream were subjected, upon the freezing properties of the ice cream mixes are shown in figure 1 and table 1.

TABLE 1

The effect of sweetened and non-sweetened frozen cream on the development of swell

TREATMENT OF CREAM	KIND OF SUGAR	RATIO OF SUGAR TO CREAM	STORAGE PERIOD OF CREAM	NUMBER OF TRIALS	TIME REQUIRED TO DEVELOP 90 PER CENT SWELL	MAXIMUM SWELL OBTAINABLE*
			hours		*minutes*	*per cent*
Not frozen	None		48	4	8	96
Frozen	None		48	4	9	94
Frozen	Sucrose	1:10	48	4	8	99
Frozen	Invert	1.33:10	48	4	8	101
			days			
Frozen	None		14	3	9	95
Frozen	Sucrose	1.5:10	14	3	7	100
Frozen	Invert	1:10	14	3	7	102
Frozen	Sucrose	4:6	14	3	7	100

* "Maximum swell obtainable" with proper consistency for drawing from freezer.

The mixes containing non-sweetened, frozen cream were slightly inferior in whipping properties to those mixes which contained non-sweetened, unfrozen cream. The swell development of the non-sweetened, unfrozen lot of cream practically coincided, during the first nine minutes of the whipping period, with that of those mixes which contained the sweetened frozen cream.

Those changes in the freezing properties of the mix, that were caused by the use of non-sweetened frozen cream, were eliminated by the addition of either sucrose or invert syrup to the cream before it was placed in frozen storage.

THE EFFECT ON THE DEVELOPMENT OF SWELL OF USING CREAM, FROZEN FOR 14 DAYS WITH OR WITHOUT ADDED SUGAR

The difference in development of swell between the mixes, which contained frozen and unfrozen cream without sugar, was smaller than the differences noted by Price and Whitaker (1). This disagreement was believed to be due to the difference in the length of time the cream remained frozen. A second series of experiments were planned to permit a longer storage period.

In this second series of experiments, a quantity of fresh, pasteurized cream, containing 47 per cent fat was divided into four portions. The first portion was not altered. To the second was added 1.33 parts of invert syrup for each 10 parts of cream by weight. To the third was added 1.5 parts of sucrose for each 10 parts of cream. The fourth portion was separated to produce 60 per cent cream and sucrose was added at the rate of 40 parts of sugar to 60 parts of cream.

All four lots of cream were placed in well-tinned ice cream cans and frozen at −15°F. After fourteen days they were thawed by immersion in cold water.

The appearance after melting of the sweetened cream in each instance was smooth and practically free from granules of milk fat. In the first series of experiments, the appearance of the cream to which the sucrose had been added, was not as smooth as that of the cream which contained invert syrup. This was apparently caused by the difference in the freezing point depression induced by the sucrose and the inverted sugar, because the granular condition was avoided by the addition of larger amounts of sucrose in this second series of experiments. That portion of the cream which contained 40 per cent sucrose was similar in body to sweetened condensed milk. It had a slightly translucent appearance and a very pleasing flavor. The non-sweetened portion presented the usual granular appearance and was the only lot to show fat separation during the pasteurizing of the mix in which it was used.

Four mixes were made which differed from each other only in that they contained a different one of the four lots of cream.

They were identical in composition to the mixes of the first series of experiments and were made and processed in the manner which has been described.

This procedure was repeated during June, 1930 until three different lots of cream had been so divided and treated.

The results of the freezing operations are summarized and presented in figure 2 and table 1.

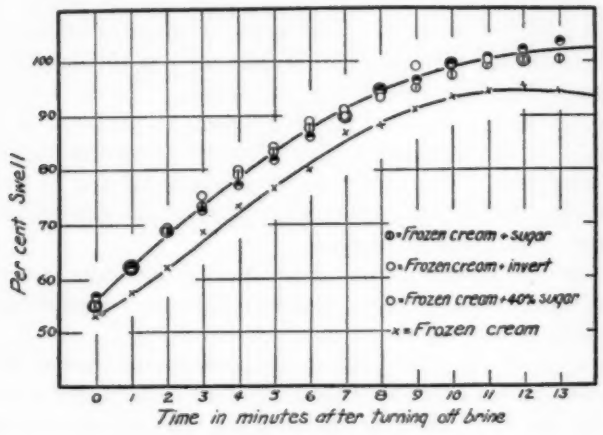

FIG. 2. THE INFLUENCE OF SWEETENED CREAM, FROZEN FOR 14 DAYS, ON THE DEVELOPMENT OF SWELL OF ICE CREAM

The swell developed by the mixes containing the sweetened, frozen cream was practically identical, regardless of the sugar content of the cream. The swell obtained in the mix containing the non-sweetened, frozen cream, developed more slowly and attained a lower maximum.

Here again is evidence of the tendency of sugar to prevent that change in frozen cream which causes a decrease in the development of swell of the ice cream mix in which it is used.

Observations indicated that the quality of the ice cream was not altered by the difference in the treatment of the frozen cream.

THE EFFECT ON THE DEVELOPMENT OF SWELL OF USING EGG YOLK FROZEN WITH AND WITHOUT SUGAR

A third experiment was planned to determine the effect on the development of swell of the mix of the use of egg yolk frozen with sugar to prevent the separation of lecithin.

The yolks of 18 eggs were mixed together and then divided into two equal portions. One portion was not altered but to the second was added 1.33 parts of invert syrup for each 10 parts of egg yolk by weight. The yolks were then placed in well-tinned cans

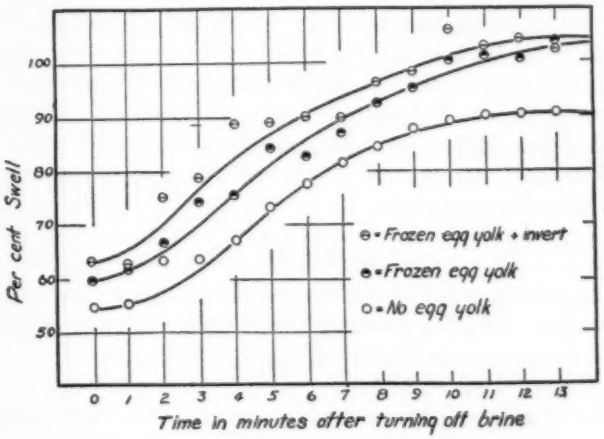

FIG. 3. THE INFLUENCE OF EGG YOLKS, FROZEN WITH INVERT SIRUP, ON THE DEVELOPMENT OF SWELL OF ICE CREAM

and frozen for forty-eight hours at −15°F. The containers were then placed in cold water until all ice disappeared.

The appearance of the sweetened egg yolks was not altered by this treatment, but the non-sweetened lot contained small, dark orange-colored jelly-like masses which could not be reincorporated by agitation.

An ice cream mix of the usual composition was made in which butter served as the source of milk fat and dry skimmilk as the source of skimmilk solids in order that no appreciable quantity of lecithin could be present in the milk products. It was divided

into three 50-pound portions. To one lot was added the non-sweetened egg-yolk. To the second was added the sweetened egg-yolk. The third portion remained unaltered. Each portion was then pasteurized, homogenized, cooled and aged in the usual manner which has been described.

The three mixes were then frozen and the results are shown in figure 3.

The swell developed by the mix which contained the sweetened egg-yolk was slightly higher than that of the mix which contained the non-sweetened egg-yolk. Both of these mixes possessed distinctly better whipping properties than the egg-free mix.

The process of freezing and thawing the egg-yolks caused a change in their influence on the development of overrun. The addition of sugar to the yolks, before they were frozen, decreased the intensity of this effect. The work of Urbain and Miller suggests that these results were due to the influence of the sugar on the lecithin of the egg-yolk.

CONCLUSIONS

Ice cream mix which contains sweetened frozen cream as the only source of milk fat possesses better whipping properties than mix which is made with non-sweetened frozen cream.

Invert syrup and sucrose are equally effective for sweetening cream for storage if the concentrations of each cause approximately the same freezing point depression in the storage cream.

The available evidence indicates a possible relationship between lecithin and the swell development of ice cream mix.

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FAT SOLUBLE VITAMINS

XXXIII. THE DETERMINATION OF VITAMIN A AND ITS STABILITY IN BUTTER FAT TO ULTRA VIOLET RADIATIONS*

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In making use of the ultraviolet rays as an antirachitic activating agent, it is necessary, of course, to consider harmful or destructive effects which may be produced simultaneously as the food materials are exposed. It is well known that protein substances become coagulated, that enzymes are destroyed and that even starches acquire a very distinctive taste upon long exposure. Zilva (1) showed that vitamin C in decitrated lemon juice was not destroyed by exposure to the radiations of a quartz mercury vapor lamp for six to eight hours; vitamin B in autolyzed yeast, after being exposed for six hours, apparently was not destroyed, but vitamin A in butter showed itself to be much more labile to this treatment, because after an exposure of eight hours, the butter fat, fed at a level of 2 per cent did not prevent the incidence of ophthalmia in rats. Spinka (2) reported that ultraviolet light did not destroy vitamin B in yeast nor vitamin C in lemon juice, but treated butter fat was found toxic unless air was excluded. Zilva (3) reported that the death of Spinka's mice possibly was not due to toxicity but to destruction of vitamin A. These deductions were based upon Zilva's experience with rats in his previous paper (1). Later Zilva (4) showed that this destruction was due to ozone generated during the course of radiation. When the radiation was carried out in CO_2 vitamin A was not destroyed. Peacock (5) and Holmes and Pigott (6) also referred to vitamin A destruction by ultra violet. Titus, Hughes, Henshaw, and Fitch (7) showed that in the irradiation of milk, vitamin A con-

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tained therein was materially decreased. Drummond (8) has been reported as having pointed out the possibility of vitamin A destruction in the course of activation of milk, and also of having called attention to the well known changes produced in palatibility. Steenbock and Coward (9) pointed out that both vitamins A and B showed remarkable stability to ultraviolet treatment. Hogan and Hunter (10) found that while vitamin B was not destroyed, the heat stable factor often accompanying it was destroyed. Supplee and Down (11) found that milk could be activated either in the dry or liquid form by a short time exposure with careful control of other conditions without destruction of vitamin A or induction of toxic effects. Steenbock and Black (12) showed that olive oil excessively irradiated lost the antirachitic activity originally induced. With cod liver oil they likewise found that after long continued exposure the oil became totally devoid of antirachitic potency. This probably explains why it is impossible (13) to increase materially the antirachitic potency of cod liver oil even though it contains activatable substances. Presumably the compounds already activated are destroyed in the same measure as the unactivatable substances become potent. Willimott and Wokes (14, 15) found that in cod liver oil destruction of vitamin A continued after irradiation.

The above resumé of the experience of various investigators shows very definitely that the induction of antirachitic activation in food materials by means of ultraviolet irradiation must be carried out very carefully. Especially is this true of such foods as milk, which due to their content of labile proteins and fats are probably as susceptible to harmful agents as any with which we have to deal.

EXPERIMENTAL

In the various June butter fat samples irradiated as described in the preceding paper of this series (16), determinations for vitamin A content were made with samples which had been irradiated for 30 minutes, 60 minutes, and 120 minutes. As will be recalled, these samples in general were irradiated with a quartz mercury vapor lamp of the Alpine Sun type, exposing the butter

fat at depths of 0.08, 0.16, 0.32, 0.64, and 1.28 cm. in bright pie tins, $7\frac{3}{4}$ inches in diameter. Due to the fact that Zilva (4) reported failure of destruction of vitamin A when the irradiation was carried out in an atmosphere of CO_2, additional samples of butter fat were prepared which were irradiated at a depth of 0.08 cm. in the presence of nitrogen, carbon dioxide, and hydrogen. These exposures were made on 9.3 cc. of butter fat measured out into a small tin pan 10.8 cm. square. This pan was slipped into a small galvanized iron chamber, provided with a quartz window. The chamber was then closed by means of a plate clamped down, air tight, on a rubber gasket. The air was displaced from the chamber by the aforementioned gases as measured out from tanks into a large rubber balloon. The carbon dioxide and hydrogen were used as obtained commercially. The nitrogen was further purified by passing it through alkaline pyrogallol.

The content of vitamin A in the butter fats was determined on the premises outlined by Steenbock and Coward (9). We used animals produced in our general stock at approximately 40 grams in weight and approximately twenty days old. The basal ration used was composed of purified casein 18, agar 2, salts 40, (17) 4, yeast 8, dextrinized starch 68. This ration, in order to endow it with antirachitic properties in excess, was irradiated in 250 grams quantities in a pan measuring 2 feet square for a period of 30 minutes. It was stirred once after 15 minutes' exposure. The irradiation was carried out at a distance of approximately 18 inches, with the same lamp as that used for the treatment of butter fat. After the animals had been on this ration for a sufficient period of time to develop ophthalmia,—which was usually in the neighborhood of five weeks, they were taken for test. At first, we used the animals by litters. When the majority of the rats in a litter had developed ophthalmia, the entire litter, irrespective of the condition of the others, was taken. However, we soon discovered that there was no greater uniformity in the reaction of individuals from the same litter than from different litters. Accordingly, for most of the experiments the animals were taken without regard to litters, as soon as they had developed the desired degree of ophthalmia. This ophthalmia

was designated as stage 3 which was characterized by a severe erythema accompanied by bareness of eyelids, occasional laceration with excessive exudate sometimes causing closure of the eyes. In order to follow the progress of the disease in its development, as well as its regression, the ophthalmias were classified by number according to severity. The progressive stages are:

0—Normal
1—Accumulation of exudate, dry or moist
2—Swollen eyelids with or without incipient bareness
3—Erythema of conjunctiva, with or without bareness, laceration or closure
4—Purulency, accompanied by closed eyes with severe swelling and occasional blindness

and the regressive changes:

3—Absence of purulency, but persistence of erythema, bareness, swelling and exudate with eyes open or closed
2—Absence of erythema, but eyes still swollen, cornea cloudy, presence of exudate, eyelids bare
1—Absence of acute reaction, but eyes slightly dirty or watery with or without protrusion of cornea and presence of scar tissue
0—Normalcy; absence of exudate, bareness of lids and scar tissue

Inasmuch as Evans and Bishop (18) have reported the persistence of cornified cells in the vaginal smear of females as a far more sensitive symptom of vitamin A deficiency, than ophthalmia, we have examined some of our animals using their technique. Our experience is limited to 13 animals. These were started on a vitamin A free diet at an age of 21 to 22 days. They developed ophthalmia after four to five weeks on this ration. When examined for the presence of cornified cells in the vaginal smear after they had been on the ration for 37 to 41 days they were still relatively immature as the vaginal orifice was found closed. They were kept under observation from 5 to 25 days, after which periods all except one had died as a result of the dietary deficiency. When the observations were started, 4 of the animals were in stage 2 ophthalmia, 6 were in stage 3, and 3 were in

stage 4. With these severe ophthalmias only 5 females showed the presence of cornified cells. Most of them had very scanty smears and later showed a definite cyclic rhythm. Shortly before the cessation of observations, 8 females had cornified cells. From this experience, limited though it is, we are inclined to believe that data on ophthalmia are more satisfactory than those obtained by Evans' technique (18). If in our experimets we made the mistake of using the females at too early an age, taking them at this age has much to commend it because when the animals are taken later, the storage reserves are frequently so high that depletion occurs relatively slowly.

For the determination of vitamin A the butter fat was for the most part fed incorporated in the ration. This procedure was felt to be justified because we obtained the total consumption records throughout the period of feeding. We have, therefore, a check on the amounts of butter fat received by the animals in the different groups. However, in some of our experiments, namely in those in which we tested out the effect of gases other than air on the preservation of vitamin A, the butter fat was measured out in small quantities in dishes,—the basal ration in all cases being fed ad libitum. In terms of percentage of the ration, butter fat irradiated for 30 minutes was fed at levels of 0.25, 0.5, 1.0 and 2.0 per cent; irradiated for 60 minutes at 2 per cent, and irradiated for 120 minutes at 1, 2, 4 and 8 per cent.

The results on ophthalmia are not all presented for the simple reason that 4 out of the series of 9, happened to be run at such a level that they alone bring out the approximate border line of persistence as contrasted with the destruction of vitamin A. In table 1, as in the other tables, the vacant line represents the line of reference with respect to the condition of the ophthalmia in the animals after two weeks on the supplemented diet.

Table 1 shows that butter fat irradiated for 30 minutes incorporated in the ration at 0.5 per cent level had appreciable quantities of its vitamin A destroyed when irradiated at depths of 0.08 and 0.16 cm. When irradiated at greater depths the rapidity in the cure of ophthalmia was not different from that effected with the feeding of untreated butter fat at the same level.

TABLE 1

Cure of ophthalmia with butter fat irradiated 30 minutes 0.5 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																												NON-IRRADIATED				
	0.08 cm.							0.16 cm.								0.32 cm.							0.64 cm.			1.280 cm.							
	Lot 45*			Lot 50				Lot 46				Lot 52				Lot 47				Lot 54			Lot 30			Lot 29				Lot 22			
0	0	3	3	3	3	2	3	0	3	0	2	3	3	2	3	3	1	3	2	3	3	3	1	3	3	1	4	3	3	2	4	3	3	3
1	0	2	3	3	3	1	3	0	1	0	2	3	3	2	3	1	1	2	1	3	3	1	0	1	3	1	3	0	1	1	3	1	3	1
2	0	3	3	3	0	3	3	0	1	0	2	0	3	2	3	0	1	†	1	1	0	1	0	1	0	0	1	0	1	†	3	1	1	1
3	†	1	3	3	0	3	3	†	†	0	2	3	3	2	3	†	0		1	1	†	1	2	1	0	0	1	0	1		3	1	1	3
4		2	†	3	0	3	2			0	2	3	3	†	3		0		1	1		1	†	†	1	0	1	3	1		1	1	1	1
5		†		3	0	1	3			0	1	3	3		†					1		1			0	0	3	†	1		1	1	1	1
6				3	3	3	†			0	3	3	†							†		1			0	0	†		1		1	1		1
7				3	3	†				†	†	3										1									1	1		
8				3	3																													

* Each column carries the data for an individual rat.

Key to ophthalmia tables. 4 = purulency accompanied by closed eyes with severe swelling and occasional blindness. 3 = absence of purulency, but persistence of erythema, bareness, swelling and exudate with eyes open or closed. 2 = absence of erythema, but eyes still swollen, cornea cloudy, presence of exudate eyelids bare. 1 = absence of acute reaction, but eyes slightly dirty or watery with or without protrusion of cornea and presence of scar tissue. 0 = normalcy: absence of exudate, bareness of lids and scar tissue.

Table 2 shows that when the butter fat was fed at a 1 per cent instead of a 0.5 per cent level as in table 1 the ophthalmias were cured in all the groups with equal efficiency. It is to be emphasized that little importance is to be attached to the persistence of stage 1 of ophthalmia because scar tissue is usually absorbed

TABLE 2

Cure of ophthalmia with butter fat irradiated thirty minutes, 1.0 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																				NON-IRRADIATED			
	0.08 cm.							0.16 cm.				0.32 cm.			0.64 cm.				1.28 cm.					
	Lot 13				Lot 19			Lot 14				Lot 15			Lot 16				Lot 17				Lot 18			
0	0	3	3	3	1	0	3	2	3	3	1	2	3	3	2	3	4	3	4	3	2	3	4	3
1	0	2	3	0	0	0	3	3	0	2	0	3	1	0	0	0	3	0	3	0	1	1	1	2
2	†	1	1	0	0	0	1	†	0	1	0	1	1	0	†	0	3	0	3	0	1	1	1	0
3		1	1	0	0	0	0		0	1	0	1	1	0		0	1	0	1	0	1	1	†	0
4		1	1	0	0	0	0		0	1	0	1	1	0		0	1	0	1	0	1	1		0
5		1	1	0	0	0	0		0	1	0	1	1	0		0	1	0	1	0	1	1		0
6		0	1	0					0	1	0	0	0	0		0	1	0	1	0	0	1		0

TABLE 3

Cure of ophthalmia with butter fat irradiated two hours, 2.0 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																												NON-IRRADIATED			
	0.08 cm.								0.16 cm.						0.32 cm.				0.64 cm.				1.28 cm.									
	Lot 7				Lot 31			Lot 37	Lot 8				Lot 32			Lot 9				Lot 10				Lot 11				Lot 12			
0	2	2	1	0	3	4	3	3	3	2	3	0	3	2	0	3	3	3	0	3	3	0	3	3	4	3	3	3	4	3	4
1	2	3	3	0	3	3	3	3	2	2	2	0	3	1	3	1	1	3	0	0	3	0	0	0	1	0	1	0	3	1	1
2	2	1	3	0	3	1	3	3	3	†	†	3	†	†	3	2	0	0	0	0	1	0	0	0	3	0	1	0	3	1	1
3	†	†	†	†	†	†	†	†	3			2			3	1	0	0	0	0	1	0	0	0	1	0	1	0	1	1	1
4									†			1			†		0	0	0	0	1	0	0	0	1	0	1	0	1	1	1

with great difficulty. Regressive stage 1 is, therefore, to be taken as of practically the same value as stage 0.

Table 3 shows that more destruction resulted when the butter fat was irradiated for two hours at depths of 0.08 and 0.16 cm. than at the greater depths. When the amount of butter fat in the ration was increased from 2 to 4 per cent (table 4) this greater

destructive action at the aforementioned depths was still definitely demonstrable.

We have also noted that when the butter fat was exposed for 60 minutes instead of 120 and fed at a 2 per cent level, the ophthalmias were cured inside of two weeks to stage 0 and stage 1, even with those samples which had been exposed at a depth of 0.08 and 0.16 cm. The detailed results of these trials are not presented.

The above results, together with those presented in the preceding paper (16), bring out convincingly that while antirachitic

TABLE 4

Cure of ophthalmia with butter fat irradiated two hours, 4 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																							
	0.08 cm.							0.16 cm.						0.32 cm.						0.64 cm.				
	Lot 23			Lot 38		Lot 42		Lot 24				Lot 43		Lot 25			Lot 44			Lot 26				
0	3	3	1	3	3	3	1	3	3	2	2	3	0	3	3	2	3	3	1	0	3	3	3	3
1	2	1	1	3	3	3	3	1	1	2	3	3	0	3	1	1	3	3	1	0	2	3	1	3
2	3	1	2	†	†	3	2	2	2	2	2	3	0	2	1	1	0	0	0	0	1	3	1	0
3	3	1	2			3	3	1	3	2	2	3	2	1	1	1	0	0	0	0	1	0	1	0
4	†	†	†			3	2	1	†	3	†	3	3	1	1	1	0	0	0	0	3	0	1	0
5						1	2	†		3		†	3	1	1	0	0				1	0	0	0
6						3	3			†			3	1	0	0	0				2	0	0	0
7						†	†						3											
8													3											
9													3											
													†											

activation is effected more pronouncedly with the butter fat exposed in thin layers, concomitantly greater vitamin A destruction also results. The significance of the observed destruction with 30 minutes' exposure at a depth of 0.16 cm. must not, however, be over emphasized, because when the amount of butter fat in the ration was doubled, sufficient vitamin A was still present to effect a rapid cure of ophthalmias.

Besides using the cure of ophthalmia as our criterion for vitamin A content of the butter fat samples, we also kept records of the growth of the animals before and after supplementation of the

basal ration. The animals were weighed once a week and their growth curves plotted as presented in charts 1 to 5. In chart 1 are presented the results obtained with butter fat irradiated at

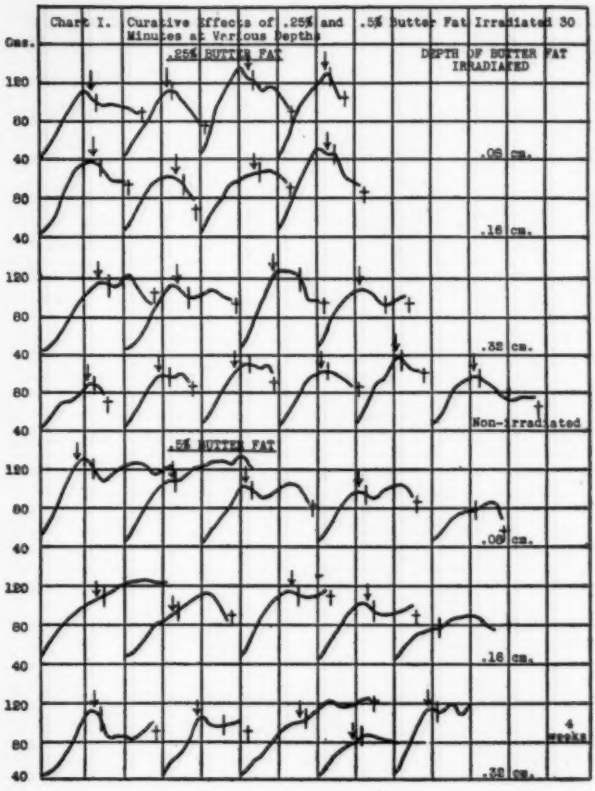

CHART 1

↓ incidence of ophthalmia, | change of ration, † death

depths of 0.08, 0.16 and 0.32 cm. when fed at levels of 0.25 and 0.5 per cent of the ration. We have not presented data on the cure of ophthalmia at the 0.25 per cent level of intake because this

was too low an intake to effect a cure even with the butter fat irradiated at the greater depths, but we have included the growth curves at this level of intake to bring out that growth was not markedly, if at all, inferior to that in the 0.5 per cent of fat. It will be recalled that on 0.5 per cent of fat, we obtained rapid cure of ophthalmia with butter fat irradiated at a depth of 0.32 cm., as contrasted with 0.08 and 0.16 cm. Inasmuch as the differences in growth do not bring out these relations we were at this point inclined to believe that the ophthalmia method of

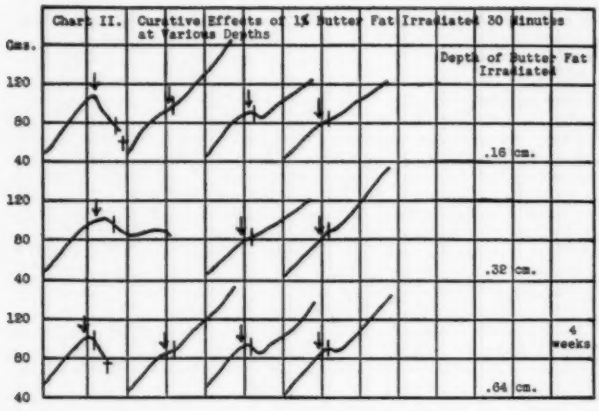

CHART 2

↓ incidence of ophthalmia, | change of ration, † death

vitamin A determination was to be preferred to the growth method. It will be noted that in the charts not all of the growth curves of the animals on which we have ophthalmia data are presented. Those shown are in every way representative of the groups as a whole.

In chart 2 are presented data with butter fat fed at a level of 1 per cent irradiated for 30 minutes at depths of 0.16, 0.32, 0.64 cm. At this level of intake the growth curves are in every way comparable to the results on ophthalmia.

In chart 3 are shown the growth curves comparing butter

fats irradiated in depths of 0.08, 0.16, and 0.32 cm. for periods of one as contrasted with two hours, fed at a 2 per cent level. When the butter fat was irradiated for one hour, growth in the animals

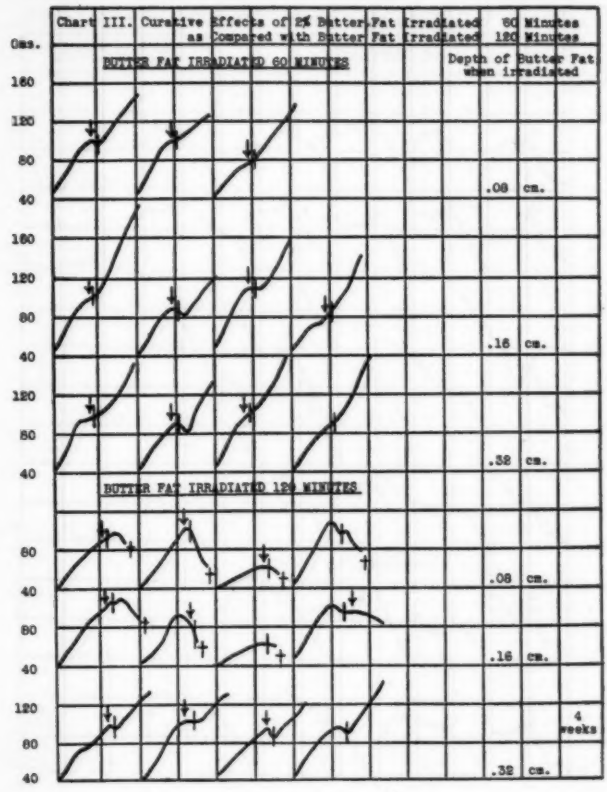

CHART 3

↓ incidence of ophthalmia, | change of ration, † death

was very promptly restored at all depths; but, when irradiated for two hours, growth was restored only with that which had been irradiated at 0.32 cm. Comparing these results with our ophthalmia data, it is significant that in this case the growth data are in

entire accord with our results on ophthalmia. By way of explaining this difference, as compared with our observations in the groups previously discussed, we are inclined to believe,—although

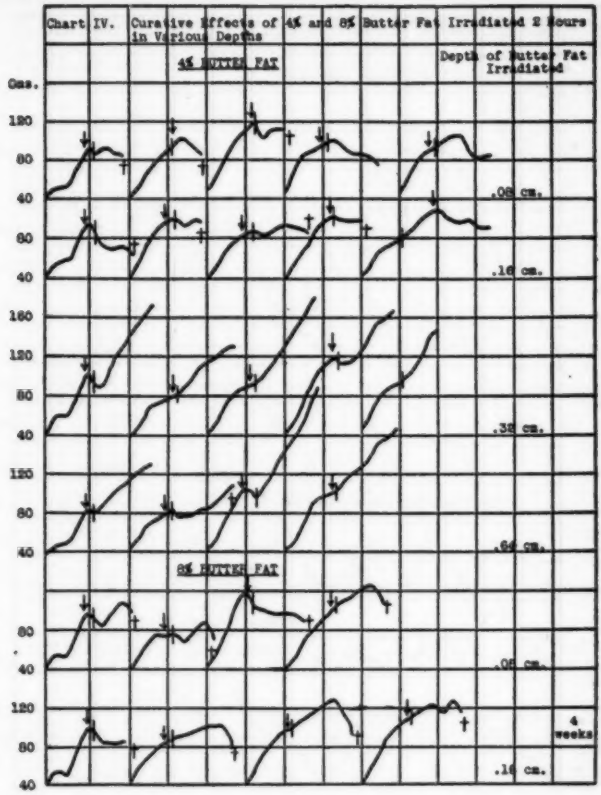

CHART 4

↓ incidence of ophthalmia, | change of ration, † death

the data selected for publication in this paper do not prove the point—that the growth observations harmonize with the cure of ophthalmia only when the loss in weight or the maintenance of constant weight previous to the change of the diet has been

TABLE 5

The average daily consumption by individual animals during the first two weeks or until death when the animal died sooner

	DEPTH OF BUTTER FAT WHEN IRRADIATED					NON-IRRADIATED
	0.08 cm.	0.16 cm.	0.32 cm.	0.64 cm.	1.28 cm.	
0.25 per cent butter fat irradiated 30 minutes						
	Lot 51	Lot 53	Lot 55			Lot 49
	grams	grams	grams	grams	grams	grams
	5.6	4.4	9.3			4.3
	5.0	4.6	6.4			6.2
	6.4	5.6	4.3			7.7
	3.2	3.0	6.7			8.4
						5.9
						3.7
Average per rat....	5.1	4.4	6.7			5.5

0.5 per cent butter fat irradiated 30 minutes									
	Lot 45	Lot 50	Lot 46	Lot 52	Lot 47	Lot 54	Lot 30	Lot 29	Lot 22
	8.9	6.9	7.1	8.4	8.5	4.7	6.1	4.9	2.9
	4.7	7.1	7.3	7.4	8.9	7.3	5.0	5.8	7.3
	6.7	4.6	6.9	5.4	4.0	7.9	8.1	4.5	8.4
		5.1	5.0	6.0	5.9			7.9	4.1
									7.4
Average per rat....	6.3		6.7		6.7		6.4	5.8	6.0

1.0 per cent butter fat irradiated 30 minutes							
	Lot 13	Lot 19	Lot 14	Lot 15	Lot 16	Lot 17	Lot 18
	3.8	5.0	3.1	3.2	1.9	4.4	6.8
	6.6	5.4	8.1	6.7	7.5	8.1	6.4
	4.9	3.9	6.4	7.4	5.7		5.0
	8.2		7.4		6.4		7.8
Average per rat....	5.4		6.3	5.8	5.4	6.3	6.5

2.0 per cent butter fat irradiated 120 minutes									
	Lot 7	Lot 31	Lot 37	Lot 8	Lot 32	Lot 9	Lot 10	Lot 11	Lot 12
	4.9	7.4	5	5.4	6.6	7.6	9.3	6.3	8.1
	3.5	4.9		2.6	2.6	6.6	5.5	8.4	5.4
	3.7	5.2		4.1	6.1	5.8	6.9	6.1	7
	1.4			6.2		7.9	7.1	8.8	8.1
Average per rat....	4.5			4.8		7.0	7.2	7.4	7.2

TABLE 5—*Concluded*

	DEPTH OF BUTTER FAT WHEN IRRADIATED								NON-IRRADIATED
	0.08 cm.			0.16 cm.		0.32 cm.	0.64 cm.	1.28 cm.	
4.0 per cent butter fat irradiated 120 minutes									
	Lot 23	Lot 38	Lot 42	Lot 24	Lot 43	Lot 25	Lot 44	Lot 26	
	gms.	*gms.*	*gms.*	*grams*	*grams*	*grams*	*grams*	*grams*	
	5.0	4.8	5.9	3.9	7.6	7.1	8.6	7.5	
	5.6	3.1	7.7	4.6	6.6	7.9	10.4	5	
	4.8			5.9		7.1	8.4	9	
				5.3		7.8		8	
Average per rat....	5.3			5.7		8.2		7.4	

N.B. To compare with ophthalmia data (tables 1 to 4), tables5 should be read taking each column in order from left to right. This will give the consumption corresponding for each rat.

of short duration. This is in accordance with the observations of Macy and co-workers (19).

Chart 4 shows that with the butter fat irradiated for two hours, fed at 4 and 8 per cent of the diet, growth was not resumed by the animals, where the butter fat had been exposed at depths of 0.08 and 0.16 cm. At greater depths, namely, 0.32 and 0.64 growth was reinitiated promptly even at the 2 per cent level of intake. This is again in entire harmony with our observations on ophthalmia, which presentation in the charts, however, is limited to the 4 per cent level of intake. The data of the 8 per cent level of intake are not presented in order to conserve space.

As previously stated, consumption records were kept in all of the foregoing experiments in order to allow us to check the intake of butter fat. In the course of our experiments we have frequently had occasion to feed a dietary supplement in portions separate from the basal ration. Under many conditions we have found this entirely satisfactory, but in other cases, we have been led to doubt the advisability of such a procedure because it gives the experimenter no guarantee that the basal ration is consumed in sufficient amounts unless this is checked up also. We have, therefore, felt that records of the total food intake are far more satisfactory. For the reader's convenience and assurance

of validity of deductions the records of food consumption for the first two weeks have been presented in table 5. While data were accumulated over the entire experimental period, it was thought

TABLE 6

Cure of ophthalmia with butter fat irradiated in air nitrogen, carbon dioxide, and hydrogen

80 mgm. daily

WEEKS	IRRADIATED IN AIR						IRRADIATED IN NITROGEN						IRRADIATED IN CO_2						IRRADIATED IN HYDROGEN						NON-IRRADIATED					
	Lot 64						Lot 65						Lot 68						Lot 70						Lot 56					
0	3	3	3	3	3	3	3	3	3	3	3	3	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
1	3	3	3	3	3	3	3	3	1	3	3	3	1	3	1	3	3	3	2	3	3	3	2	2	0	1	0	0	0	0
2	†	2	3	3	3	3	3	3	2	3	†	3	3	0	3	3	3	3	1	3	2	2	†	2	0	0	0	0	0	0
3		2	3	†	†	†	†	3	0	3		†	2	0	3	3	3	3	1	3	3	1		2							
4		†	†					3	0	3			1	†	†	3	3	3	1	3	†	†		1							
5								3	0	3			1			3	3	3	1	3				†							
6								†	0	2			†							†											
7									0	1																					
8										1																					
										†																					

320 mgm. daily

WEEKS	IRRADIATED IN AIR				IRRADIATED IN NITROGEN						IRRADIATED IN CO_2						IRRADIATED IN HYDROGEN					NON-IRRADIATED					
	Lot 66				Lot 67						Lot 69						Lot 71					Lot 72		Lot 59			
0	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3
1	3	3	3	3	2	3	1	3	0	3	0	3	3	2	3	1	3	0	3	1	1	1	1	0	1	0	0
2	3	3	3	2	0	0	0	1	0	3		2	1	0	1	0	2	0	1	0	0	1	1	0	1		
3	3	2	3	2				1		1		0	1		1	0	2	0	1								
4	2	†	†	2									1				1										
5	3			†													1										
6	3																										
	†																										

sufficient to present only the first two weeks because with a satisfactory consumption for this period the animal should have responded to the dietary supplement if it contained a sufficiency of vitamin A.

Comparing the results of our previous experiments (16) with the present, it appears that, approaching the point of maximum activation of butter fat, there becomes incident the destruction of demonstrable amounts of vitamin A. To determine if this is avoidable by eliminating oxidation, butter fat was irradiated in air, nitrogen, carbon dioxide, and hydrogen at a depth of 0.08 cm. Two hours was selected as the time of exposure, this being sufficient to effect complete destruction of vitamin A as revealed in the previous experiments. The butter fat in these experiments was fed separately in small containers, the basal ration being available for ad libitum consumption. In one series, animals were given 80 mgm. butter fat daily, and in the other 320 mgm.

Table 6 shows the results obtained with both levels of intake. It is evident that irradiation in air as contrasted with irradiation in nitrogen, carbon dioxide, and hydrogen, was more destructive, but on the other hand, preparations made in this manner compared with untreated butter fat showed that destruction of vitamin A was not prevented. In these experiments we also kept a record of the growth response of the animals, which gave results in entire harmony with the cure of ophthalmia. In order to conserve space these data were not charted.

In our experiments we did not obtain any evidence of toxicity of irradiated butter fat as reported by Spinka (2). It is well known that with excessive irradiation not only does there result complete destruction of vitamin A, but also bleaching and transformation of butter fat to a tallowy substance. When the exposure is continued to such extremes, it is, of course, entirely possible that not only unpalatability, but substances inimical to the welfare of the animal are produced as well. Willimott and Wokes (14, 15) claimed that, in cod liver oil, destruction of vitamin A continued after irradiation. While this is a possibility in view of the fact that ultra violet treatment is known to produce ozonides, yet in our experiments we failed to find any demonstrable decrease in vitamin A with irradiated butter fat stored for a period of seven months (chart 5). The storage conditions selected were those of room temperature and ice box temperature. The butter fat used for these experiments was irradiated at a

depth of 0.16 cm. and for 30 minutes under the Alpine Sun Lamp as previously described. These samples were fed in comparison with non-irradiated butter fat kept under the same conditions.

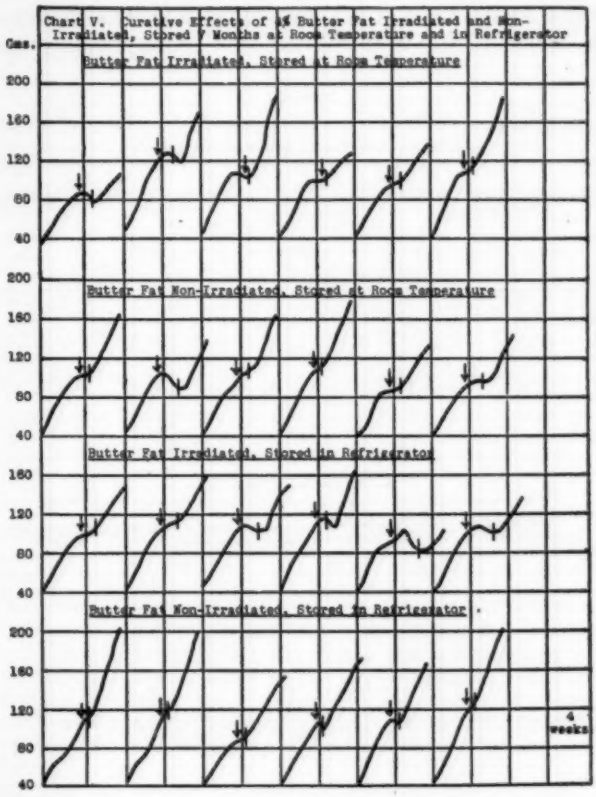
Chart V. Curative Effects of 4% Butter Fat Irradiated and Non-Irradiated, Stored 7 Months at Room Temperature and in Refrigerator
Gms.
Butter Fat Irradiated, Stored at Room Temperature
Butter Fat Non-Irradiated, Stored at Room Temperature
Butter Fat Irradiated, Stored in Refrigerator
Butter Fat Non-Irradiated, Stored in Refrigerator
200
160
120
80
40
4 weeks

CHART 5

↓ incidence of ophthalmia, | change of ration, † death

Levels of intake were 1 and 4 per cent. To insure the animals getting a sufficient intake of the butter fat, records were kept throughout the experimental period.

Table 7 and chart 5, which present the data on ophthalmia and growth respectively, show that when stored at room temperature after irradiation for this comparatively long period, there had resulted little destruction of vitamin A. These changes must be interpreted in the light of the consumption records presented in table 8. In this table it is seen that the average consumption of

TABLE 7

Cure of ophthalmia with butter fat irradiated and non-irradiated and stored under various conditions 1 per cent in ration

WEEKS	IRRADIATED, STORED AT ROOM TEMPERATURE										NON-IRRADIATED, STORED AT ROOM TEMPERATURE						IRRADIATED, STORED IN ICEBOX						NON-IRRADIATED, STORED IN ICEBOX					
	Lot 74						Lot 81				Lot 76						Lot 78						Lot 80					
0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
1	3	2	2	3	3	1	3	3	3	2	0	0	0	1	2	2	0	2	2	0	1	2	2	3	3	0	1	1	0
2	1	†	0	3	2		3	3	1	0	0	0	0	0	1	0	0	0	2	0	1	†	0	0	3	0	0	0	0
3	1		0	3	0		†	2	1	0	0	0	0	0	1	0	0	0	2	0	1		0	0	0	0	0	0	0
4			0	0	0			1	†	0									0				0						
								†																					

4 per cent in ration

WEEKS	IRRADIATED, STORED AT ROOM TEMPERATURE						NON-IRRADIATED, STORED AT ROOM TEMPERATURE						IRRADIATED, STORED IN ICEBOX						NON-IRRADIATED, STORED IN ICEBOX								
	Lot 73						Lot 75						Lot 77						Lot 79						Lot 82		
0	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	4	3	4	3	3	3	3
1	0	1	2	0	0	0	0	3	2	0	0	0	1	0	1	2	2	1	2	1	2	2	2	1	0	1	0
2	0	0	1	0	0	0	0	3	1	0	0	0	1	0	0	1	0	1	1	0	1	0	1	0	0	0	0
3	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0

some of the groups receiving the stored butter fat was slightly lower than the others. The difference in intake, however, was not of such an order as to be outside of experimental error, either due to variation in the groups of animals or accuracy of the experimental method when carried out under the best of conditions as recognized at the present time.

TABLE 8

Average daily consumption of individual animals during first two weeks or until death of animal died before being two weeks on supplemented ration. (Butter fat stored seven months)

1 per cent in ration

	IRRADIATED, STORED AT ROOM TEMPERATURE										NON-IRRADIATED, STORED AT ROOM TEMPERATURE					
	Lot 74						Lot 81				Lot 76					
Grams consumed...	8.1	1.5	5.6	7.6	7.0	6.3	3.9	6.0	6.3	7.8	10.4	6.9	9.1	7.2	7.5	8.3
Average.....	6.0										8.2					

	IRRADIATED, STORED IN ICEBOX							NON-IRRADIATED, STORED IN ICEBOX						
	Lot 78							Lot 80						
Grams consumed...	7.8	6.0	6.4	8.5	6.5	4.7	6.1	8.2	8.2	9.1	9.9	6.1	7.3	
Average.....	6.6							8.1						

4 per cent in ration

	IRRADIATED, STORED AT ROOM TEMPERATURE						NON-IRRADIATED, STORED AT ROOM TEMPERATURE					
	Lot 73						Lot 75					
Grams consumed...	6.6	8.0	10.4	8.3	9.3	10.6	9.1	6.2	8.9	10.3	9.0	9.4
Average.....	8.9						8.8					

	IRRADIATED, STORED IN ICEBOX						NON-IRRADIATED, STORED IN ICEBOX									
	Lot 77						Lot 79						Lot 82			
Grams consumed...	8.4	8.5	7.8	7.8	4.6	6.9	11.5	10.6	8.6	9.3	9.4	12.0	11.4	9.9	13.6	
Average.....	7.3						10.7									

SUMMARY

Different techniques for determination of vitamin A have been discussed. The Evans-Bishop technique was found to have no advantages over those techniques which depend upon the reinitiation of growth or the cure of ophthalmia. Of these latter the

ophthalmic method was preferred, because of the specific nature of its reaction, and its rapid responsiveness. The growth method was also found reliable and correlated closely in results obtained with the ophthalmic method when the animals were taken for test shortly after growth ceased. Furthermore, with animals of variable weight we experienced difficulty in deciding just when they should be taken for the growth test.

Vitamin A is destroyed by ultraviolet treatment even when the irradiation is carried out in an atmosphere of CO_2, nitrogen, or hydrogen. However, these gases apparently do retard the destructive reaction somewhat. It is still possible that our preparations of butter fat may have contained a sufficiency of oxygen in solution to have allowed destruction by oxidation. This hypothesis appears tenable when one takes into consideration the very small amounts of vitamin A which can produce cure of ophthalmia or reinitiation of growth.

The destruction of vitamin A was found to be proportional to the time of exposure and inversely proportional to the thickness of the layer of fat exposed.

The irradiation necessary to secure maximum activation was found to cause some destruction of vitamin A. Practically, however, maximum activation may not be desirable from the dietetic standpoint, so that the antirachitic activation of butter fat by direct irradiation is a practical possibility without danger of destroying vitamin A.

Butter fat, antirachitically activated, was found to have retained most of its vitamin A even after storage for seven months at room temperature.

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THE RELATION OF CERTAIN PLANT PROCESSES TO FLAVOR DEVELOPMENT IN MARKET MILK*

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There are various marketing operations necessary to give raw milk a desirable form and place utility. To determine the relationship existing between certain of these processes and the flavor development in market milk, this study was made.

MILK GRADING

To encourage the production of milk relatively free from bacteria many milk plants have adopted systems of grading, whereby the producers are rewarded or penalized according to certain bacterial standards. That buying milk on a bacterial grade basis will enable the plant operator to develop a raw milk supply of relatively low bacterial content is evidenced by the record of milk grading at the University of Illinois Creamery for the year ending September 1, 1928. During this period milk was purchased from forty to forty-five producers who averaged about 100 pounds each daily. A bacterial count was made on all milk once each week, using the Official Plate Method. A monthly summary of the record of the results is shown in table 1. The data show that 34.48 per cent of all deliveries investigated had a count not exceeding 50,000 bacteria per cubic centimeter, while only 34.92 per cent of all deliveries had a count of over 200,000 bacteria per cubic centimeter.

This record indicates that most of the milk delivered was relatively low in bacteria. However, as it was thought possible to make further improvements, a system of farm inspection was adopted. A bacterial count was made once a week (twice a week

* An abstract of a thesis submitted by P. H. Tracy in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Dairy Husbandry in the graduate school of the University of Illinois, 1930. Received for publication October 10, 1930.

in summer) upon each patron's milk and in case the results indicated some neglect at the farm the University representative immediately called and conferred with the producer in regard to possible causes of the trouble.

In addition, during June, July, and August, a temperature standard was established in order to encourage proper cooling of the milk at the farm. Any milk having a temperature of 70°F. or over when delivered at the Creamery was penalized one grade, the lowest of the four grades being excepted.

TABLE 1

Summary of bacterial counts on raw milk delivered to the university creamery

Percentage of all deliveries containing following bacterial counts

MONTH	1-10 THOUSAND BACTERIA PER CUBIC CENTIMETER		10-50 THOUSAND BACTERIA PER CUBIC CENTIMETER		50-200 THOUSAND BACTERIA PER CUBIC CENTIMETER		200-500 THOUSAND BACTERIA PER CUBIC CENTIMETER		OVER 500 THOUSAND BACTERIA PER CUBIC CENTIMETER	
	1927-1928	1928-1929	1927-1928	1928-1929	1927-1928	1928-1929	1927-1928	1928-1929	1927-1928	1928-1929
September	2.24	30.95	14.54	50.00	19.48	11.90	34.16	2.39	29.38	4.76
October	0.00	28.08	56.80	58.00	25.00	11.60	6.80	2.32	11.40	0.00
November	0.00	46.94	36.32	34.70	29.50	14.28	16.00	4.08	18.18	0.00
December	0.00	40.00	31.20	42.00	37.70	14.00	15.55	2.00	15.55	2.00
January	0.00	63.26	51.10	32.65	37.20	4.09	7.05	0.00	4.65	0.00
February	2.27	32.65	72.70	53.06	22.76	10.20	0.00	4.09	2.27	0.00
March	0.00	26.10	31.70	54.34	41.46	17.39	9.77	2.17	17.07	0.00
April	0.00	31.26	55.00	45.82	40.00	12.50	0.00	10.42	5.00	0.00
May	0.00	2.22	16.60	15.54	39.00	40.06	23.80	26.64	20.60	15.54
June	2.44	6.51	31.70	28.21	29.27	41.41	9.75	15.19	26.84	8.68
July	0.00	0.00	2.38	18.56	26.18	34.92	19.04	34.92	52.40	11.60
August	0.00	2.50	6.56	22.50	19.68	55.00	21.34	20.00	52.42	0.00
Average	0.58	25.87	33.88	37.95	30.60	22.28	13.60	10.35	21.31	3.55

A summary of the bacterial checks for the year beginning September 1, 1927, and ending August 31, 1929 is given in table 1. It is plainly evident that the grading program followed the second year was more effective in securing a raw milk supply of a low bacterial content than was that followed during the preceding twelve months. The average percentage of total deliveries having a count less than fifty thousand bacteria per cubic centimeter was increased from 34.48 to 63.82, while the percentage of

total deliveries having a count over two hundred thousand was reduced from 34.92 to 13.90.

THE TEMPERATURE STANDARD

Before the development of the Methylene Blue Reduction test for bacteria in milk, it was often the practice to establish a temperature standard during the summer months in order to force producers to properly cool their milk. However, with the adoption of the bacterial standards the temperature factor in many cases was no longer considered. A study made during August in a milk plant handling about fifty thousand pounds of milk daily, the milk being graded on the Methylene Blue Reduction test, is of interest in this connection.

A summary of the data shows that of the lots of milk having a temperature of 70°F. or above when received at the plant, 39.4 per cent were Grade A, 45.4 per cent were Grade B, and 13.7 per cent were Grade C. Of those having a temperature below 70°F., 53.6 per cent were Grade A, 43.5 per cent were Grade B, and 2.9 per cent were Grade C. Seventy-six per cent of all the milk received had a temperature of 70°F. or over. Considering all the milk received, 70.1 per cent of the Grade A, 76.7 per cent of the Grade B, and 93.5 per cent of the Grade C milk had a temperature of 70°F. or over.

In a further study made at the University Creamery, it was noted that during the month of May, 1929, much of the raw milk was being delivered at a temperature over 70°F. During the last week in May the results of one day's temperature check showed that only three out of forty-four deliveries had a temperature below 70°F. The minimum temperature of the milk when received was 60°F., and the maximum temperature was 86°F. Since warm milk at the time of delivery is undesirable, the temperature standard referred to above was made effective June first. All milk having a temperature of 70°F. or over at the time of delivery (morning's milk included) was penalized one grade, the fourth or lowest grade being excepted. A summary of the data showing the relation between the milk grade and the temperature during June, July, and August is given in table 2.

It appears from these data that a temperature standard in conjunction with a bacterial standard will assist in improving the quality of milk through the encouragement of proper cooling. However, it will be noticed that the temperature of most of the milk as it reached the plant was such that, considering its age and possible bacterial seeding, bacterial growth would have been rapid had the milk not been immediately cooled or pasteurized.

For some reason farmers find it possible to produce milk of reasonably low bacterial content without paying much attention to cooling. Unpublished data by Prucha and Tracy show that this is possible when milk is handled in sterile utensils. Milk drawn from the udder of a cow directly into sterile glass tubes

TABLE 2

Relation between milk grade and temperature

BACTERIA PER CUBIC CENTIMETER	NUMBER OF DELIVERIES	PERCENTAGE OF DELIVERIES	AVERAGE TEMPERATURE	MAXIMUM TEMPERATURE	MINIMUM TEMPERATURE	PERCENTAGE UNDER 70°F.
			°F.	°F.	°F.	
Under 50,000	249	53.5	67.2	75	56	81
50,000–200,000	124	29.1	68.8	76	62	62
200,000–500,000	58	13.6	71.0	77	59	41.4
Over 500,000	16	3.7	69.4	80	56	37.5

has been kept at 68°F. for fifteen hours without any appreciable increase in bacteria. It seems logical to conclude, therefore, that the low bacterial counts in the milks mentioned in the above studies were due to extensive use of chemical sterilizers on the part of the farmers, most of whom were known to use this method of utensil disinfection.

It is evident that the milk grading program followed at the University Creamery has resulted in a raw milk supply of very low bacterial content. In turn it might be concluded that the quality of the milk was very high, since so much emphasis has been placed upon bacterial content that to some extent we have come to think of milk quality as being measurable by the amount of bacterial contamination. To be sure, only a small proportion of the milk received during the period of this study was of poor flavor due to bacterial action. Yet examination of a number of

these lots of milk has shown that the official "yardstick," which specifies a bacterial standard only, is incomplete. Cowy, barny odors in the raw milk were common; puckery flavors were present in most samples; a few were tallowy, and some were metallic. On several occasions, milk was received which had a medicinal flavor.

PASTEURIZATION

The main purpose of pasteurization is to destroy bacteria present in milk. Incidently, however, the process results in the elimination of some of the undesirable flavors. Several separate deliveries of milk were pasteurized in glass bottles and cooled in the same container. The flavor of the raw milk varied considerably. It was noticed with the exception of some feed flavors that in practically all cases the barn flavors in the raw milk were partially or completely eliminated by pasteurization. Puckery flavors as well as those of a medicinal nature were not appreciably benefited by the heat treatment.

Continued holding of the milk at pasteurizing temperature should not be practiced because of the cooked flavor which results. In one experiment milk was placed in quart bottles which in turn were placed in a hot water bath in which the milk was heated to 142°F. At the end of every thirty minutes for a period of six hours a bottle was removed, and after cooling in cold water, it was placed in a 40°F. room. After twenty-four hours all the samples were judged for flavor. The samples removed at the end of thirty and sixty minutes were comparable. However, the third sample, the one held at 142°F. for ninety minutes had a cooked flavor. This cooked flavor defect became more noticeable in each successive sample, the one held six hours having a very pronounced cooked flavor. In batch pasteurization it is a good practice to drop the temperature of the milk in the vat to about 125°F. if the bottling will require much more than thirty minutes.

RELATION OF CERTAIN METALS TO FLAVOR IN MILK

Milk is usually exposed to metal surfaces from the time of its removal from the udder of the cow until it is placed in the glass

or paper bottle in which it is marketed. Some studies have been made of the effect of dissolved metals upon the flavor of milk. In 1905 Golding and Feilman (1) found that milk passed over a partially detinned copper cooler acquired a metallic flavor in about eighteen hours. Later, various other investigators (2, 3, 4, 5) showed that the presence of certain metals in milk resulted in the development of a flavor defect described as tallowiness. In general, the metals that showed a corrosive action were the ones that were found to impart undesirable flavors to the milk products.

In order to study the relation of metals to flavor in milk, various metals were added in small quantities to milk before pasteurization. Milk was also pasteurized in the presence of small strips of different kinds of metals. Various flavor defects resulted, such as puckeryness, metallic flavors, and tallowiness, described by some writers as a pasteboard flavor. Iron, copper, German silver, and Benedict nickel were particularly harmful in this respect. Aluminum, tin, nickel and Allegheny metal had the least effect upon the milk flavor.

Lactic acid, caustic and sodium hypochlorite filtrates of tin, iron, lead, aluminum, copper, iron oxide, copper oxide, and aluminum oxide when added to milk affected its flavor. Of the metals studied, copper and iron were the most detrimental to milk flavor. Pasteurization of milk containing these filtrates was not found to be a factor in flavor development, nor was the fullness of the bottle of much significance.

RELATION OF DAIRY EQUIPMENT TO TALLOWY FLAVOR IN MILK

As has been shown, tallowiness is very likely to result from the presence of certain metals in milk. Tallowiness in dairy products is so called because of its resemblance to the flavor and odor of spoiled tallow. Holm and Greenbank (6) attribute the defect to an autoxidation of the butterfat, the reaction being hastened by such factors as heat, light, moisture, acidity, and enzyme action. These investigators also state that as a result of the oxidation the oleic acid radical is split, resulting in the formation of aldehydes and acids which possess the tallowy odor. Hunziker and Hos-

man (7), however, concluded "that tallowiness is not the direct result of oxidized oleic acid, but rather is caused by products formed in the butter during the absorption of oxygen by the oleic acid, such as glycollic acid ester of oleic acid."

It seems from these studies that in the processing of market milk the one factor most likely to produce a tallowy flavor is the presence of certain metals, which may be added to the milk through its contact with utensils and equipment. Tin has been used commonly as a lining for milk cans, vats, coolers, and other equipment and is itself nearly harmless to the flavor of milk. Unfortunately the tinned surface does not always remain, and as it is gradually removed through wear, copper or iron surfaces are usually left exposed. As has been shown these two metals are very detrimental to milk flavor.

It is to be expected that any plant process which produces a corrosion of the metal linings of the different dairy utensils and equipment will indirectly contribute to a flavor defect of market milk. The results of Hunziker (8), et al., and Prucha, Tracy, and Tuckey (9) show that sodium hypochlorite solutions containing as little as 35 p.p.m. of chlorin and 0.5 per cent solutions of sodium hydroxide or trisodium phosphate will corrode all the metals ordinarily used in dairy equipment, with the exception of the polished chromium metals. It seems likely, therefore, that much of the raw milk entering the plant contains a certain amount of dissolved metal and that more is added in the handling of the milk in the plant. There would be an accumulative effect of the metal so that the development of a flavor defect, such as tallowiness, might be due to more than one source of metal contamination.

RELATION OF FAT CONTENT OF MILK TO TALLOWY FLAVOR

In order to study the relation of the relative percentage of fat in milk to the effect of added metal upon the flavor, samples of milk were prepared containing 4.9, 4, 3, 2, 1, and 0 per cent of butterfat. To each lot of milk a small quantity of a lactic acid solution of copper oxide was added. The samples were held at 40°F. for seventy-two hours before being judged. In the absence

of fat the copper salt caused a metallic flavor, but with the fat present a tallowy flavor resulted. As the fat content of the milk was increased the tallowy flavor became more apparent.

CONTAMINATION OF MILK AT THE FARM WITH METALS

It is possible that by the time the raw milk arrives at the plant it will contain variable amounts of iron or copper salts. Small amounts of these metal salts probably could not be measured accurately, but by the addition of a definite quantity of a copper salt to equal portions of different deliveries, differences in the flavor developing might be explained through differences in the total amount of iron or copper salts present. Accordingly, several samples of raw milk were treated in this way. Each of the raw milks was tested for chlorin, using the method devised by Rupp (10), and the bacterial count of each was determined. After pasteurization in glass bottles the milk was divided into two parts. To one part a small quantity of a copper salt was added. The samples were all stored at 40°F. for forty-eight hours before being judged. The results showed a marked difference in the way in which milks produced by different farmers (proper allowance being made for variations in butterfat) were affected by the addition of a small amount of copper salt.

In several instances the control samples—those to which no copper had been added—had a noticeable tallowy flavor after being held for forty-eight hours at 40°F. The majority of the producers supplying the milk used in this experiment were using a sodium hypochlorite sterilizer at the time of the experiment (July). The tests for chlorin suggest that chemical sterilizers had been used for sterilizing some of the equipment at the farm. It seems reasonable to conclude that in some cases at least an appreciable amount of copper or iron salts gets into the raw milk through the farm utensils.

CONTAMINATION OF MILK AT THE PLANT WITH METALS

a. Relation of tinned-copper internal tubular cooler to tallowiness

The passage of milk through the plant is accomplished by means of glass and metal equipment. From the data and discussion

already presented in regard to the effect of certain metals upon milk flavor, one would expect market milk quality to be impaired as a result of contamination by vats and other equipment made of metals which are unsuitable either because of their composition or because of the treatment they have received. Milk certainly should not be permitted to come into contact with either iron or copper. Plants using either strong alkali solutions or sodium hypochlorite mixtures on tinned equipment will eventually have trouble from tallowiness caused by the metals left exposed by the corrosion of the tin.

Such a situation was encountered at the University Creamery. An unclean, tallowy flavor was noticed in the pasteurized milk, especially during the winter months. At the bottle filler the milk flavor seemed satisfactory, yet numerous complaints were received from customers that the milk had a very undesirable flavor. On investigation, it was noticed that the customers making the complaints were being served from the milk cases that were the last to be removed from the refrigerated room. This milk was the first milk cooled each day. In order to determine whether there was a difference in the quality of the milk bottled first and that bottled last the following experiment was performed:

Three quarts each of the first bottled and last bottled milk from the first batch was saved on Tuesday, Thursday, and Saturday of each week for a period of six weeks. The samples were stored at 40°F. and were judged after two, four, and six days. The results are given in table 3.

The milk (usually about two hundred gallons) was pasteurized in a glass lined vat and forced by air through two sections of 1.5 inch internal tubular coolers 12 feet long with 12 tubes in each section. The construction of the tubes was tin on copper. Various types of chemical sterilizers had been used in disinfecting the coolers, and some copper was exposed. The coolers were washed and rinsed usually in the afternoon and were disinfected the following morning just before using, with a chlorin solution containing 50 to 75 p.p m. of chlorin.

In an experiment made as a preliminary to the one summarized

TABLE 3

Flavor development in first and last milk bottled—flavor criticism

AFTER 2 DAYS		AFTER 4 DAYS		AFTER 6 DAYS	
First bottled	Last bottled	First bottled	Last bottled	First bottled	Last bottled
Tallowy	Good	Tallowy	Slight tallowy	Very tallowy	Slight tallowy
Slight tallowy	Good	Slight tallowy	Good	Slight tallowy	Good
Very tallowy	Good	Very tallowy	Good	Very tallowy	Good
Very tallowy	Good	Very tallowy	Tallowy	Very tallowy	Tallowy
Tallowy	Good	Tallowy	Good	Tallowy	Good
Slight tallowy	Good	Slight tallowy	Good	Slight tallowy	Good
Very tallowy	Good	Very tallowy	Slight tallowy	Tallowy, puckery, bitter	Slight tallowy
Very tallowy, slight medicinal	Slight tallowy	Very tallowy	Tallowy	Very tallowy	Tallowy
Tallowy	Good	Tallowy	Good	Very tallowy	Slight tallowy
Tallowy	Good	Very tallowy	Slight tallowy	Very tallowy	Good
Very tallowy	Good	Very tallowy	Tallowy	Very tallowy	Tallowy
Slight tallowy	Good	Slight tallowy	Good	Tallowy	Good
Slight tallowy	Good	Slight tallowy	Good	Tallowy	Good
Tallowy	(No sample)	Tallowy	Slight tallowy	Very tallowy	Tallowy
Tallowy	(No sample)	Tallowy	(No sample)	Tallowy	(No sample)
Tallowy	(No sample)	Tallowy	(No sample)	Very tallowy	(No sample)
Slight tallowy	Good	Slight tallowy	Slight tallowy	Slight tallowy	Slight tallowy

in table 3, an attempt was made to avoid the development of the tallowy flavor by pumping a thousand pounds of rinse water through the coolers after disinfecting with the chemical sterilizer. This was apparently of no advantage. The use of the chemical sterilizer was then discontinued altogether, but the tallowy flavor still persisted.

The tallowy flavor described in these experiments seemed to be due primarily to the presence in the milk of dissolved metals, possibly copper oxide. Probably the first milk to pass through the coolers received most of the metal oxide, whereas the last to pass through was only slightly contaminated.

b. Benedict nickel internal tubular coolers as a source of off-flavors in market milk

The unsatisfactory results obtained with the tinned copper internal tubular coolers led to their replacement with tubes made from Benedict nickel, an alloy of nickel, copper, and zinc. Soon after the installation of this equipment a peculiar, unclean, tallowy flavor was noted in some of the milk twenty-four to forty-eight hours old. In order to locate the source of this flavor defect, the following experiments were made:

The mixed herd milk of the regular run (about 250 gallons) was used in this experiment. The milk was pumped through an internal tubular preheater, the inner tubes of which are made of Benedict nickel, into a vat lined with Alleghany metal. After pasteurization some of the milk was cooled in a well-tinned bucket submerged in cold water. Some of this milk was then transferred to a glass bottle. About two quarts of the hot milk were then pumped through the pump (a brass piston pump) regularly used to force the milk from the vat through the coolers. After cooling in a tin bucket, a quart of this milk was placed in a glass bottle. The remaining milk was then pumped through the cooler into the bottle filler, a sample of the first milk discharged at the end of the cooler being saved. The first and last bottles of milk bottled were also saved. The results are given in table 4.

It was evident that although the pump was a possible source

of tallowy flavor,[1] the internal tubular coolers were a more important factor in contributing to the tallowy flavor in the milk. The flavor defect due to the use of internal tubular coolers was similar to that caused by the partially detinned copper tubes previously referred to, but the milk usually had an additional

TABLE 4

Comparison of samples taken at different points in the cooling process—flavor criticism

SAMPLE	A		
	After 7 hours	After 24 hours	After 48 hours
Directly from vat	Slight puckery	Slight puckery	Slight puckery
First through pump	Slight metallic	Slight tallowy	Slight tallowy
First through cooler	Watery, unclean sterilizer flavor	Tallowy, watery, unclean	Very, very tallowy
First bottle at filler	Fairly good	Tallowy	Tallowy
Last bottle at filler	Fairly good	Slight tallowy	Slight tallowy
	B		
	After 7 hours	After 24 hours	After 120 hours
Directly from vat	Good	Good	Old flavor
First through cooler*	Watery, unclean, sterilizer flavor	Watery, unclean, sterilizer flavor, puckery	Watery, puckery, very tallowy
First through pump	Slight metallic, puckery	Metallic, puckery	Metallic, tallowy
First bottle at filler	Fair to good	Slight tallowy	Tallowy
Last bottle at filler	Good	Slight tallowy	Tallowy

* A Mojonnier test on the milk from the vat showed a fat content of 4.06 per cent, that from the pump tested 3.85 per cent, while the first from the cooler tested 3.63 per cent. This dilution was in spite of efforts made to drain the system of rinse water.

flavor described as unclean. Examination of a large number of samples of the first and last milk bottled clearly showed that the first milk through the coolers was of poorer quality than the last

[1] It is interesting to note that a small lot of milk (about 1 gallon) pasteurized in the presence of the removable parts of the Simplex brass pump developed a very strong tallowy flavor in twenty-four hours, whereas the control sample was of good quality.

milk cooled. There was a variation in the seriousness of the defect from day to day, yet the flavor would invariably develop in time. Peculiarly the trouble was much more noticeable in winter than in summer. The off-flavor would not develop until after the milk had been stored at 40°F. for twenty-four to forty-eight hours. Contrary to expectation the flavor developed faster in milk stored at 40°F. than that stored at 68°F. The flavor developed regardless of whether steam or chemical sterilizers were used on the equipment.

In order to obtain further evidence that the internal tubular coolers were the source of the off-flavor, the following experiment was performed over a period of several days:

The raw milk to be used for bottling was pumped directly from a well-tinned dump vat into the Allegheny steel vat. After pasteurizing at 142°F. for thirty minutes the milk was cooled in the following ways:

Cooling method number	
1	Dipped from vat and poured over surface cooler no. 1
2	Dipped from vat and poured over surface cooler no. 2
3	Dipped from vat and poured over surface cooler no. 3
4	Dipped from vat and poured over surface cooler no. 4
5	Pumped from vat over surface cooler no. 1
6	Pumped from vat over surface cooler no. 2
7	Pumped from vat over surface cooler no. 3
8	Pumped from vat over surface cooler no. 4
9	Pumped through water section of internal tubular coolers and then poured over surface cooler no. 2
10	Pumped through both sections of internal tubular coolers
11	Dipped into glass bottle and cooled in bottle
12	Control—pasteurized and cooled in bottle

In the case of cooling methods nos. 1 to 9, 10 gallons of the milk were used. In the case of method no. 10, almost 100 gallons of milk were used. In the case of methods 11 and 12, 1 quart of milk was used. In all cases the first bottles cooled were saved for judging. The samples were judged after twenty-four and forty-eight hours, and the study was continued for one week. The results of one day's run are recorded in table 5.

It was evident from the data that the flavor of the milk may

be affected by surface as well as by internal tubular coolers. The latter, however, seemed to be a more serious cause of an unclean and very often tallowy flavor. To be sure, the milk cooled in glass was not always perfect in flavor, indicating metal contamination of the milk prior to its being placed in the vat, a condition made more serious, however, by the subsequent contamination from the milk pump and coolers.

TABLE 5

Relation of method of cooling to tallowy flavor—flavor criticism

COOLING METHOD NUMBER	AFTER 24 HOURS	AFTER 48 HOURS
1	Metallic, unclean	Tallowy
2	Puckery but clean	Puckery
3	Metallic, unclean	Metallic, unclean
4	Slight metallic	Slight metallic
5	Tallowy	Tallowy
6	Slight tallowy	Slight tallowy
7	Unclean, metallic, puckery	Unclean, metallic, puckery
8	Metallic, puckery	Metallic, puckery
9	Unclean, puckery	Unclean, tallowy, puckery
10	Very unclean, puckery	Tallowy, puckery, unclean
11	Slight puckery	Puckery, slight unclean
12	Slight puckery but best of all samples	Slight unclean

EFFECT OF SUNLIGHT UPON MILK FLAVOR

That light hastens the development of a tallowy flavor in butter fat has been known for some time (11). Hammer and Cordes (12), however, were the first to report a tallowy flavor in market milk due to sunlight. Skim milk when exposed to sunlight, according to these investigators, developed an abnormal flavor that was not tallowiness. By the use of brown milk bottles they were able to prevent the occurrence of either of these two defects.

Frazier (13) explains the action of light as a catalyst in the oxidation of milk fat. He also suggests that a metal catalyst would assist the light in hastening the oxidation of the butterfat.

Schulz (14) states that the deterioration of the taste produced

by ultra violet irradiation of milk is caused by the action of hydrogen peroxide on the proteins. Tiede and Reyher (15) concluded that the action was due to the activity of ozone.

In order to further study the significance of diffuse light and sunlight in their relation to milk flavors, a series of experiments were performed exposing milk varying in composition and amount of metal contamination to light rays under variable conditions.

It was found that two flavor defects resulted from the exposure of milk to sunlight, one a tallowiness and the other a burnt flavor. When the milk was exposed to the diffuse light only a tallowy flavor developed. The formation of this flavor was hastened by the presence of metals. The burnt flavor tended to mask the tallowiness when present and seemed to be due to an action upon the milk serum rather than the fat, as the defect was more noticeable in skim milk than in whole milk. There was no direct relation between the burnt flavor formation and the presence of copper salts.

RELATION OF THE USE OF CHEMICAL STERILIZERS TO FLAVOR IN MARKET MILK

Dairy utensils and equipment are treated in various ways in order to destroy the bacteria present. For many years hot water and steam alone were used for this purpose. Recently, however, chemical sterilizers have been extensively employed in the disinfection of both farm and plant equipment. There are various types of chemical sterilizers, but the ones ordinarily used for dairy utensils are those containing chlorin, such as the hypochlorites of calcium and sodium and organic compounds such as sodium-sulphorichloramid, more commonly called chloramine-T.

Prucha (16) has made extensive studies of the application of chemical disinfection to the dairy industry and concludes that chlorin compounds are suitable sterilizers for utensils. He warns against treating the milk with chemical sterilizers in order to destroy bacteria.

The amount of information in the literature relative to the effect upon milk flavor of the use of chemical sterilizers in disinfecting dairy utensils is limited. A series of experiments were performed, therefore, to study this factor.

It was found that chemical sterilizers added either directly to the milk or through treatment of the bottle affected the flavor of milk. A puckery, burning sensation usually resulted. When stronger concentrations of the sterilizing solutions were used, a sickening, unclean flavor resulted. Whether the sodium hypochlorite was alkaline or slightly acid did not seem to be of any importance from the standpoint of the effect upon flavor.

It was also found that the addition of sodium hypochlorite to milk caused a more pronounced tallowiness to develop in the samples containing a sufficient amount of metal to produce tallowiness, indicating an additional effect of the disinfectant. It was also noted that in most cases in which a tallowy flavor developed in milk exposed to metal strips, the defect was more pronounced in the milk brought into contact with the strips previously treated with a sodium hypochlorite solution.

An occasional occurrence of a medicinal flavor has been noted in the raw milk received at the University Creamery. A few complaints were received that the pasteurized milk "tastes as if it had medicine in it." No information could be found in the literature as to the cause of this defect.

Numerous attempts to reproduce the medicinal flavor by the direct addition of the sterilizer failed to result in anything more than the defects noted above. A series of experiments was next performed in which the milk was exposed to glass and metal surfaces treated with chemical disinfectants.

It was found that the defect was due to bringing milk into contact with tinned utensils and equipment treated with a chemical sterilizer a short time before using. If the tinned surface was allowed to dry before adding the milk or if the equipment was used immediately after treating with the chlorin compound, the defect usually did not occur.

According to Buswell (17) in the chlorination of city water supplies the presence of phenol is intensified by the addition of chlorin. The phenol flavor is not the same as the medicinal flavor discussed previously. Recently, Mattick (18) studied the cause of phenol flavor in sterilized milk and concluded it was due to bacterial action. According to Bell (19), p-cresol may be

derived from the bacterial degradation of tyrosine, which can yield either p-cresol or phenol.

It was found that the addition of sodium hypochlorite to milk containing a small quantity of phenol greatly intensified the flavor. Since small quantities of phenols may be absorbed by the milk either from the air or by direct contact with contaminated utensils, and since phenol may result from bacterial action, the addition of small quantities of a chlorin sterilizer may result in a noticeable phenol flavor in the market milk, which otherwise might have escaped detection.

SUMMARY

The relation between a number of plant processes and the quality of market milk as indicated by its flavor has been studied. In the absence of more suitable methods the flavor of the milk was determined organoleptically.

One of the most important of the plant processes bearing upon the quality of market milk is the use of an effective grading system. Such a system should provide for bacterial, flavor, and temperature control in the raw milk up until the time of delivery.

Pasteurization improves the flavor of most raw milk. Continued heating at 142°F. for over one hour, however, may result in a noticeable cooked flavor.

Milk should not be brought into contact with either iron or copper surfaces. Benedict nickel also may have a detrimental effect upon milk flavor. The development of a tallowy flavor in milk is closely associated with metal contamination. Glass and Allegheny metal have practically no ill effects on milk flavor.

Two kinds of flavor defects may occur in milk exposed to sunlight, one a tallowiness, and the other a burnt flavor. Diffuse light is an important factor in the development of tallowy flavor in milk, especially that containing an added copper salt.

The use of chlorin sterilizers on farm and plant equipment should be confined to those of a non-corrosive nature. In no case should the disinfectant be added directly to the milk, and all utensils and equipment treated with chlorin sterilizers should be rinsed with uncontaminated water before adding the milk.

Otherwise, puckery, unclean, or medicinal flavors may develop. The odor and flavor of phenol, when present in milk is greatly intensified by the presence of chlorin in the form of sodium hypochlorite.

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CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS

IV. THE ASSIMILATION OF CALCIUM FED AS CALCIUM GLUCONATE*

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INTRODUCTION

Recent interest in gluconic acid has brought about improved methods of production (1) and developed uses for its calcium salt, in which form it is chiefly obtained. The calcium salt, because of its solubility, lack of taste, and other valuable qualities, has been found advantageous for use in calcium therapy (2). For this reason it seemed of interest to determine whether a compound of this particular type (containing five hydroxyl-groups) could be readily assimilated from the alimentary tract and so prove a valuable source of supplementary calcium in the ration of lactating cows. Accordingly a balance experiment was undertaken to investigate this point.

EXPERIMENTAL

Two pure-bred, three-year old, Holstein cows, in their first lactation were placed in the metabolism stalls on November 30, 1929. Cow 212 had calved August 7, 1929, and was giving 21 kgm. of milk per day. Cow 213 had calved August 13, 1929, and was giving 19 kgm. of milk per day.

The cows were given a basal ration consisting of United States Grade No. 1 alfalfa hay and a grain mixture made up as follows: wheat bran, 30; corn meal, 40; soy bean meal, 20; linseed meal, 10; and sodium chloride, 1. This was fed in such amounts as the cows would clean up and in sufficient quantity to more than satisfy their nutritive requirements, according to the standards of Savage (3).

* Received for publication October 29, 1930.

The experiment was divided into four three-week periods. The basal ration supplemented with calcium gluconate (5 per cent of the grain) was given during the first three weeks of the experi-

TABLE 1

Percentage composition of feeds

PERIOD	FEED	H_2O	Ca	P	N
December 6 to January 10.....	Alfalfa hay I	12.10	1.300	0.241	2.58
January 11 to February 11....	Alfalfa hay II	11.91	1.345	0.246	2.61
February 12 to March 2.....	Alfalfa hay III	11.39	1.339	0.255	2.58
December 6 to 16.....	Grain 60 G I	11.40	0.580	0.737	3.14
December 17 to 27.....	Grain 60 G II	11.60	0.570	0.747	3.11
December 28 to January 8.....	Grain 60 I	11.20	0.149	0.753	3.22
January 9 to 17.....	Grain 60 II	11.10	0.157	0.751	3.30
January 18 to 30.....	Grain 60 G III	11.15	0.586	0.697	3.07
January 31 to February 7.....	Grain 60 G IV	12.53	0.458	0.717	3.18
February 8 to 19.....	Grain 60 L I	12.23	0.561	0.745	3.20
February 20 to March 2.....	Grain 60 L II	12.34	0.555	0.717	3.12

TABLE 2

Percentage composition of milk

WEEK	COW 212			COW 213		
	Ca	P	N	Ca	P	N
December 9 to 15.....	0.108	0.101	0.528	0.120	0.094	0.502
December 16 to 22.....	0.113	0.097	0.534	0.122	0.091	0.498
December 23 to 29.....	0.111	0.104	0.544	0.120	0.091	0.506
December 30 to January 5.....	0.105	0.106	0.550	0.114	0.089	0.484
January 6 to 12.....	0.114	0.102	0.547	0.117	0.096	0.500
January 13 to 19.....	0.108	0.104	0.531	0.110	0.095	0.490
January 20 to 26.....	0.114	0.105	0.522	0.119	0.092	0.505
January 27 to February 2.....	0.107	0.106	0.528	0.118	0.096	0.492
February 3 to 9.....	0.109	0.104	0.546	0.116	0.091	0.485
February 10 to 16.....	0.114	0.104	0.539	0.121	0.089	0.506
February 17 to 23.....	0.114	0.101	0.539	0.121	0.095	0.502
February 24 to March 2.....	0.114*	0.104*	0.540*	0.115	0.094	0.498

* Sample lost, arbitrary figures used.

ment. (Feeding began three days previous to the collection of excreta.) The basal ration alone was given during the second three weeks. During the third three weeks calcium gluconate was

TABLE 3

Weekly calcium balances

WEEK	cow 212					cow 213				
	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield
Calcium gluconate supplement										
	grams	*grams*	*grams*	*grams*	*kgm.*	*grams*	*grams*	*grams*	*grams*	*kgm.*
December 9 to 15	991.4	161.0	1184.4	+32.0	149.8	829.9	160.4	1025.2	+34.9	133.7
December 16 to 22	1045.1	165.1	1180.8	−29.4	146.1	884.9	164.7	1021.7	−27.9	135.0
December 23 to 29	1065.3	158.5	1178.1	−45.7	142.8	817.6	161.0	967.1	−11.5	134.2
No supplement										
December 30 to January 5	781.2	148.8	912.9	−17.1	141.7	547.0	142.4	622.9	−66.5	124.9
January 6 to 12	771.6	158.8	857.0	−73.4	139.3	589.8	159.7	676.5	−73.0	136.5
January 13 to 19	765.2	156.5	905.5	−16.2	144.9	635.1	150.5	747.0	−38.6	136.8
Calcium gluconate supplement										
January 20 to 26	978.1	170.0	1114.4	−33.7	149.1	883.5	157.9	987.3	−54.1	132.7
January 27 to February 2	933.2	160.4	1076.5	−17.1	149.9	881.5	160.0	977.0	−64.5	135.6
February 3 to 9	1018.5	156.6	1123.1	−52.0	143.7	785.7	153.6	915.6	−23.7	132.4
Limestone supplement										
February 10 to 16	1123.7	171.5	1183.2	−112.0	150.4	872.0	154.2	971.8	−54.4	127.4
February 17 to 23	1023.3	169.2	1180.3	−12.2	148.4	804.0	153.8	969.3	+11.5	127.1
February 24 to March 2	1056.3	167.6*	1177.7	−46.2	147.0	836.2	151.7	966.9	−21.0	131.9

* Sample lost, arbitrary figure used.

again added to the ration, and during the fourth and last three weeks limestone, supplying an equivalent quantity of calcium, was substituted for the calcium gluconate in the grain mixture.

The supplemented ration was fed first, preceding the feeding of the basal ration unsupplemented, thus reversing the usual pro-

TABLE 4

Weekly phosphorus balances

WEEK	cow 212				cow 213			
	P in urine and feces	P in milk	P in feed	P balance	P in urine and feces	P in milk	P in feed	P balance
Calcium gluconate supplement								
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
December 9 to 15	408.2	151.3	616.1	+56.6	391.0	125.7	569.0	+52.3
December 16 to 22	467.9	141.7	619.7	+10.1	420.1	122.9	572.4	+29.4
December 23 to 29	476.6	148.5	622.4	−2.7	446.8	122.1	565.4	−3.5
No supplement								
December 30 to January 5	469.6	150.2	626.2	+6.4	423.9	111.2	489.7	−45.4
January 6 to 12	457.7	142.1	575.5	−24.3	425.4	131.0	531.0	−25.4
January 13 to 19	425.6	150.7	588.1	+11.8	438.1	130.0	541.1	−27.0
Calcium gluconate supplement								
January 20 to 26	365.4	156.6	533.5	+11.5	384.1	122.1	510.8	+ 4.6
January 27 to February 2	383.3	158.9	530.4	−11.8	435.4	130.2	512.4	−53.2
February 3 to 9	437.8	149.4	586.6	−0.6	406.5	120.5	522.0	−5.0
Limestone supplement								
February 10 to 16	471.8	156.4	606.3	−21.9	429.0	113.4	539.9	−2.5
February 17 to 23	441.7	149.9	604.3	+12.7	389.1	120.7	530.9	+21.1
February 24 to March 2	450.2	152.9*	592.2	−10.9	409.5	124.0	526.4	−7.1

* Sample lost, arbitrary figure used.

cedure. In this way any favorable effect of the supplement upon calcium assimilation was enabled to operate while the milk yield was at its maximum and the drain upon calcium resources greatest. According to the usual practice where the supplement follows a period on a basal ration, if the balances improve, as the

experiment proceeds and the milk yield declines, it is uncertain whether the improved balances are attributable to the effect of the declining milk yields or to the supplement administered.

The alfalfa hay was chopped and stored in sacks. Three

TABLE 5

Weekly nitrogen balances

WEEK	cow 212				cow 213			
	N in urine and feces	N in milk	N in feed	N balance	N in urine and feces	N in milk	N in feed	N balance
Calcium gluconate supplement								
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
December 9 to 15	2570.4	790.9	3603.6	+242.3	2359.8	671.2	3231.9	+200.9
December 16 to 22	2711.3	780.2	3592.8	+101.3	2405.5	672.3	3221.6	+143.8
December 23 to 29	2687.4	776.8	3584.7	+120.5	2234.1	679.1	3110.6	+197.4
No supplement								
December 30 to January 5	2749.2	779.4	3654.0	+125.4	1884.5	604.5	2745.1	+256.1
January 6 to 12	2593.6	762.0	3386.8	+31.2	2262.5	682.5	2993.1	+48.1
January 13 to 19	2561.6	769.4	3509.6	+178.6	2305.9	670.3	3126.9	+150.7
Calcium gluconate supplement								
January 20 to 26	2382.6	778.3	3243.0	+82.1	2311.7	670.1	2998.1	+16.3
January 27 to February 2	2458.7	791.5	3200.3	−49.9	2355.5	667.2	3006.9	−15.8
February 3 to 9	2532.8	784.6	3558.7	+241.3	2070.1	642.1	3059.7	+347.5
Limestone supplement								
February 10 to 16	2719.2	810.7	3561.3	+31.4	2228.8	644.6	3063.6	+190.2
February 17 to 23	2584.4	799.9	3538.0	+153.7	2084.0	638.0	3043.4	+321.4
February 24 to March 2	2593.1	793.8*	3503.6	+116.7	2164.4	656.9	3011.4	+190.1

* Sample lost, arbitrary figure used.

batches of hay and 9 batches of grain were used during the experiment. The cows were fed hay and grain twice each day and milked three times a day. They were not pregnant and were not bred during the experiment. They were weighed on the first

three days of each month and the weights averaged. The average weights (kilograms) for the months, December, 1929, January, February and March, 1930 were, respectively for cow 212—487, 502, 510, 527; for cow 213—490, 486, 504, 517; showing a steady gain in weight throughout the experiment.

The Ca/P ratio in the feed ranged mostly between 1.75 and 2.00 in the supplemented periods and between 1.25 and 1.50 in the unsupplemented period.

Table 1 shows the percentage composition of the feeds used and table 2 the percentage content of calcium, phosphorus and nitrogen in the milks. Tables 3, 4 and 5 give the calcium, phosphorus, and nitrogen balance data.

DISCUSSION

The cows sustained their milk yields remarkably well, one cow giving more during the last three weeks of the experiment than during the first three. Whether this is to be attributed to an inherent characteristic of the animals, to the effect of the calcium supplements which they received, or to some other factor is not known. Sanders claims that a cow's milk yield is best sustained during the first lactation (4).

The blood-plasma calcium for the two cows 212 and 213 rose from 9.3 and 9.1 mgm. respectively before the experiment to 10.1 and 9.6 mgm. two weeks after the experiment began. The calcium level at the end of the experiment was 9.9 and 10.2 mgm. respectively. This may have some significance.

A casual inspection of the calcium balances shows that both cows retained calcium during the first week. This may be explained by the suddenly increased calcium intake with the introduction of calcium gluconate and the lag in excretion of the former low-calcium ration. Another factor is the somewhat depleted state of the cows which had been milking for nearly four months when the experiment started. This is particularly true of cow 213 which had received less than her nutritive requirements for three of the four months and which displayed a greater average calcium assimilation throughout the experiment.

The fourth and the seventh weeks, when changes in calcium

intake were made, might be expected to show the effect of lag in excretion, but the effect does not seem to be apparent here. Probably the somewhat depleted condition of the cows is a more important factor in explaining the positive calcium balances in the first week than the effect of lag in excretion.

The calcium balances are only slightly less negative when calcium gluconate was given than when no supplement was given indicating little value for a calcium supplement and the results in the comparison of calcium gluconate and limestone as supplements are contradictory, indicating no advantage of either one over the other.

The milk contained more calcium during the periods when a supplement was given.

The strongly positive phosphorus balances in the first week indicate that the retention of calcium during this week has permitted the simultaneous retention of phosphorus to restore depleted bone tissue.

The phosphorus balances, like the calcium, are less negative when calcium gluconate was given than when no supplement was given. The phosphorus balances show the same contradictory results in the comparison of calcium gluconate and limestone supplements, indicating no superiority of one over the other.

The nitrogen balances indicate a greater retention of nitrogen by cow 213, which may be explained by the fact that she was slightly underfed previous to the experiment.

The response which a cow exhibits to any given change in ration is doubtless dependent largely on her state of nutrition. Thus in the present experiment cow 213 shows smaller losses of calcium and greater gains in nitrogen than cow 212. She even shows a retention of calcium while receiving the limestone supplement. Cow 213 was somewhat undernourished when the experiment began. This serves to emphasize the necessity for care in the standardization of animals for nutrition experiments.

CONCLUSION

A supplement of calcium gluconate was of little value in improving the calcium and phosphorus balances of cows giving 19 to 21 kgm. of milk daily.

A supplement of limestone gave inconclusive results.

We are indebted to Drs. H. T. Herrick and O. E. May of the Bureau of Chemistry and Soils for the calcium gluconate used in this experiment.

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THE GERMICIDAL ACTION OF RAW MILK UPON AN ORGANISM OF THE SWEET CURDLING TYPE*

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The fact that fresh, raw milk exerts a bactericidal or antiseptic action upon many bacteria is well established. Investigators who have studied this phenomenon in its relation to the keeping quality of milk generally agree that the germicidal property is of academic interest only and without significant influence upon the general nature of the changes taking place in the milk. It is generally supposed that the destructive or inhibiting action is entirely too feeble and transitory to be of any practical importance.

In the course of a study upon the growth of bacteria in milk an active sweet curdler was encountered which proved to be unusually sensitive to the bactericidal action of raw milk. The restraining action of several samples of mixed milk was sufficiently strong and prolonged to suggest that this factor may, under certain conditions, play an important rôle in influencing the quality of the milk. The sweet curdling culture was isolated from milk about three months previous to this study; it was a rapidly growing Gram-positive sporulating rod, with optimum temperature about 37°C.

In a preliminary experiment with the sweet curdler the raw Holstein milk was found to be more susceptible to coagulation than the raw Jersey milk. The samples were taken from two cans of the mixed milk of each breed chosen at random from a supply of about 15 cans. The following day the experiment was repeated and the result was similar, the variation being only in the degree of difference in the coagulation time. These observations suggested the presence of a germicidal substance in the Jersey milk.

* Received for publication November 3, 1930.

The thermolabile nature of the germicidal substance in milk indicated a means of ascertaining the cause of the delayed coagulation in these experiments. One portion of the divided samples from each breed was heated in the Arnold sterilizer for fifteen minutes. This temperature is in excess of that required to completely inactivate the germicidal substance. The heated and unheated portions were then adjusted to a temperature of 37°C. after which each was inoculated with equal quantities of a broth culture of the sweet curdler. When this procedure was followed, the raw milks curdled in the same order and at about the same intervals as before, but the heated Jersey sample curdled shortly before the heated Holstein sample. This result was considered to be conclusive evidence of the presence of a germicidal substance in the Jersey milk.

In order to learn something of the extent to which the raw Jersey milk affected the growth of the sweet curdler the following experiment was carried out. Fifty cubic centimeter samples of the thoroughly mixed milk of each breed was divided into two equal portions and put into separate sterile flasks. One portion received the heat treatment already described, the other was held untreated. The heated and raw samples were then immersed in a warm water bath and held until the temperature of the milk reached 37°C. All the samples were then inoculated with a small drop of an eight-hour broth culture of the sweet curdler. After these were thoroughly agitated the microscopic count was determined, following which the flasks were placed in an incubator at 37°C. Observations for coagulation were made at hourly intervals and counts repeated at the end of four and eight hours. The direct microscopic method of enumeration was employed in preference to the plate method for the reason that consistent plate counts could not be obtained with the test organism. This difficulty is not infrequently encountered in plating aerobic spore formers. The direct microscopic count also served to minimize the error introduced into the plate method by the tendency of *Streptococcus lactis* to grow in pairs and chains. In most of this work the milk was five to six hours old at the time the samples were taken. Occasionally the samples represented a mixture of the morning's and previous night's milking.

Table 1 shows the results obtained in one of these experiments. After inoculation each sample contained about the same number of the test organisms. At the end of five hours the Holstein raw milk contained twice as many of these organisms as the Jersey raw sample. At the 9½-hour period the ratio was considerably reduced, indicating that the inhibiting agent was less effective. In this experiment, however, a slight difference was still manifest at the end of twenty-three hours. During the first nine hours the rate of growth in the inoculated raw and heated Holstein samples was quite similar, though the development was always somewhat greater in the latter. After twenty-four hours this difference was

TABLE 1

Growth of the sweet curdler in raw and heated Holstein and Jersey milk, and time of coagulation

SAMPLE	BACTERIA PER CUBIC CENTIMETER				COAGULATION TIME
	0 hours	5 hours	9½ hours	23 hours	
					hours
Holstein, raw*	65,400	11,550,000	43,000,000	86,800,000	6
Holstein, heated	64,900	12,600,000	47,600,000	131,600,000	5¼
Jersey, raw†	64,100	5,250,000	40,810,000	82,000,000	9½
Jersey, heated	65,200	11,620,000	46,970,000	91,000,000	5½

* Plate count before inoculation 670.

† Plate count before inoculation 1030.

usually much greater, although this result was somewhat variable. Coagulation of the Jersey raw milk was delayed three and one-half hours in this experiment. The data would indicate that coagulation was not entirely dependent upon numbers, as the Holstein sample curdled with much fewer organisms than the corresponding Jersey sample. Differences in the salt content (buffer action) of the milk of the two breeds may have exerted some influence upon the coagulation time.

In table 2 are recorded the results of another experiment in which only raw milk was used. Divided samples from two cans were inoculated with the sweet curdler alone, and with a similar quantity of both the sweet curdler and *S. lactis*. Counts were made only upon the sweet curdler. At the end of three hours,

both Holstein samples contained nearly four times as many organisms as the corresponding Jersey samples. At four hours the ratio was over 5:1. In eight hours the inhibiting effect was less pronounced. The marked restraining action evident here is reflected

TABLE 2

The sweet curdler grown independently and in combination with S. lactis in raw Holstein and Jersey milk, and time of coagulation

MILK	INOCULUM	NUMBER OF SWEET-CURDLING BACTERIA PER CUBIC CENTIMETER				COAGULATION TIME
		0 hours	3 hours	4 hours	8 hours	
						hours
Holstein	Sweet curdler	28,000	1,040,000	3,400,000	30,000,000	4½
Holstein	Sweet curdler and *S. lactis*	28,200	1,120,000	3,000,000	26,000,000	4½
Jersey	Sweet curdler	25,000	296,000	640,000	14,200,000	10½
Jersey	Sweet curdler and *S. lactis*	26,100	226,000	620,000	15,200,000	10½

Holstein count before inoculation 5000.
Jersey count before inoculation 2100.

TABLE 3

The sweet curdler and S. lactis grown in combination in raw and heated Holstein and Jersey milk, and time of coagulation

SAMPLE	BACTERIA PER CUBIC CENTIMETER								COAGULATION TIME
	0 hours		4 hours		8 hours		22 hours		
	Sweet curdler	*S. lactis*	Sweet curdler	*S. lactis*	Sweet curdler	*S. lactis*	Sweet curdler	*S. lactis*	
									hours
Holstein, raw*	29,000	448,000	8,300,000	21,700,000	19,880,000	27,100,000	94,500,000	939,000,000	4½
Holstein, heated	29,300	378,000	9,200,000	21,000,000	26,600,000	44,200,000	180,000,000	1,260,000,000	4½
Jersey, raw†	33,000	350,000	968,000	19,148,000	8,820,000	31,000,000	121,800,000	327,600,000	9½
Jersey, heated	30,000	500,000	9,960,000	22,260,000	26,600,000	22,820,000	242,200,000	1,026,900,000	4

* Plate count before inoculation 330.
† Plate count before inoculation 1700.

in the relative coagulation times, the difference being over six hours. Under these conditions the sweet curdler was not appreciably affected by association with *S. lactis*.

In table 3 another series of results is presented. In this experi-

ment both the sweet curdler and *S. lactis* were inoculated into the raw and heated milks of both breeds. The pronounced restraining action of the raw Jersey milk upon the sweet curdler was evident after four hours, the ratio of the numbers of the sweet curdler in the Holstein and Jersey milks at this time was more than 9:1. In eight hours, though the ratio was considerably less, the Holstein milk still contained twice the number of test organisms present in the Jersey milk. At twenty-two hours a difference was still evident. The growth of the *S. lactis* in the raw milks was quite similar. There appeared to be a slight restraining action upon these organisms during the first four hours, but at the end of eight hours in this experiment there were slightly more *S. lactis* in the Jersey raw than in the Holstein raw. In general agreement with the results in table 1, the sweet curdler developed somewhat more rapidly in the heated milks during the first eight hours, but at twenty-four hours the difference was much greater. In this experiment there was a difference of five hours in the curdling time with raw milks. There appears to be somewhat better correlation between the number of organisms and the coagulation time here, as coagulation apparently occurred in both samples when the numbers were in the vicinity of 10 million. The coagulation of the heated milks was in the usual order and at about the same intervals.

DISCUSSION

Experimental evidence suggesting that the germicidal phenomena may be of general commercial importance in milk production is quite in harmony with some of our modern practices. Throughout the New York Milk Shed and in many other parts of the United States, morning's milk is never cooled before delivery to the milk plant. The night's milk is often cooled by submerging the warm milk in 10-gallon cans in ice water. Under these conditions the cooling takes place rather slowly. It has been shown by Marquardt and Dahlberg (1) that these practices are possible only because of the germicidal property of the milk.

It should be observed that the germicidal property of the milk in these studies was determined at 37°C., the optimum tem-

perature for the test organism. Hunziker (2) and other investigators have shown that the germicidal phenomena is most evident at about 21°C., although the effect is of longer duration at still lower temperatures. In view of this fact it seems quite probable that a more pronounced effect would have been observed at lower incubation temperatures. The fact that the samples in this study were all taken from full 10-gallon cans of milk, representing the milk of from 3 to 6 cows, would suggest that the germicidal substance was present in relatively high concentrations in most of the Jersey milk for, if only present in the milk of a few cows, the effect would have been greatly reduced or lost by the high dilution. It is true that a restraining action, even of the intensity found in these milks, would under ordinary conditions have little influence in maintaining a low total bacterial count due to the fact that the usual predominating types of Micrococci, *S. lactis*, and others are only slightly affected. Nevertheless, when the milk supply is subject to heavy contamination with organisms of the sweet curdling type, and is subsequently held at relatively high temperatures for several hours, a strong germicidal action may have an important protective influence. This would be true especially during the hot summer months when temperature conditions for this type of organism are near the optimum and opportunity for contamination of all kinds is very great. When milk produced under these conditions is condensed, evaporated, or otherwise utilized within four to eight hours a strong bactericidal action may be instrumental in the prevention of heavy spoilage losses.

SUMMARY

Certain samples of mixed raw milk exerted a marked restraining action upon an organism capable of rapidly producing a sweet curdling type of fermentation. This action was present in the milk over a period of several months.

Coagulation produced by the sweet curdler was delayed from three and one-half to six hours in different samples of mixed raw milk.

Coagulation usually occurred when the sweet curdler attained a population of from 10 to 40 million.

The marked germicidal activity in this study was confined to Jersey milk.

Under the conditions of these experiments, *S. lactis* exerted but little influence upon the development of the sweet curdler and was only slightly restrained by the bactericidal activity of the milk.

Both *S. lactis* and the sweet curdler multiplied more rapidly in the heated milk than in the non-germicidal raw milk. The difference was relatively small during the first eight hours, but thereafter was usually large.

These observations indicate that under certain conditions a strong germicidal action may be of distinct value in protecting the milk supply.

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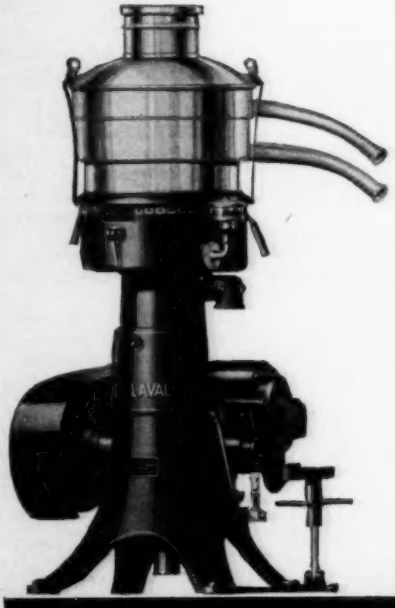

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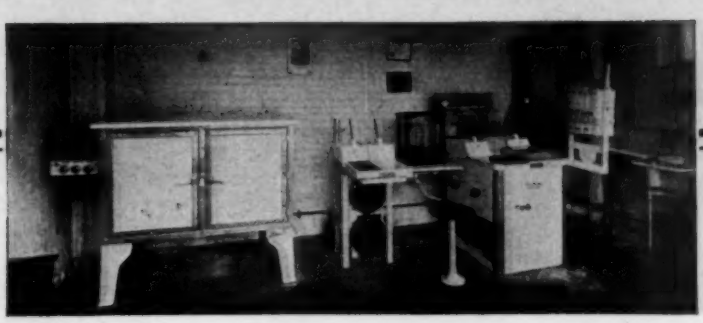

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